

Title: NUCLEIC ACID AND CORRESPONDING PROTEIN  
ENTITLED 101P3A41 USEFUL IN TREATMENT AND  
DETECTION OF CANCER  
First Inventor: Daniel E. H. AFAR, et al  
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Figure 1

GATCAAACCTTTCCATTCAAGAGTCCTCTGATTCAAGATTTAAATGTTAACATTGGAGACAGTTACAGAAAAAAATTCC  
TTAATAAAAATACAACCTCAGATCCTCAAATATGAAACTGGTTGGGGAACTCTCATTTCATTTCAATATTATTCTCTTGTGTTTC  
TTGCTACGTATAATTATTAATTCCTGACTAGGTGTTGGAGGGTTATTACTTTCAATTTCATTTTACCATGCAAGTCCAAATCTAAAC  
TGCTTCTACTGATGGTTACAGCATTCTGAGATAAGAATGGTACATCTAGAGAACATTGCCAAAGGCCTAACACAGCAAAGGAA  
AATAAACACAGAAATAAATAAAATGAGATAATCTAGCTTAAACTATACTTCTCTTTAGAACATCCCAACACATTTGGATC

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FIG. 2A

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9	18	27	36	45	54
5' CAG AGA GGC TGT ATT TCA GTG CAG CCT GCC AGA CCT CTT CTG GAG GAA GAC TGG					
-----	-----	-----	-----	-----	-----
63	72	81	90	99	108
ACA AAG GGG GTC ACA CAT TCC TTC CAT ACG GTT GAG CCT CTA CCT GCC TGG TGC					
-----	-----	-----	-----	-----	-----
117	126	135	144	153	162
TGG TCA CAG TTC AGC TTC <u>ATG</u> ATG GTG GAT CCC AAT GGC AAT GAA TCC AGT					
-----	-----	-----	-----	-----	-----
M	M	V	D	P	N
171	180	189	198	207	216
GCT ACA TAC TTC ATC CTA ATA GGC CTC CCT GGT TTA GAA GAG GCT CAG TTC TGG					
-----	-----	-----	-----	-----	-----
A	T	Y	F	I	L
225	234	243	252	261	270
TTG GCC TTC CCA TTG TGC TCC CTC TAC CTT ATT GCT GTG CTA GGT AAC TTG ACA					
-----	-----	-----	-----	-----	-----
L	A	F	P	L	C
333	342	351	360	369	378
CTT TGC ATG CTT TCA GGC ATT GAC ATC CTC ATC TCC ACC TCA TCC ATG CCC AAA					
-----	-----	-----	-----	-----	-----
L	C	M	L	S	G
387	396	405	414	423	432
ATG CTG GCC ATC TTC TGG TTC AAT TCC ACT ACC ATC CAG TTT GAT GCT TGT CTG					
-----	-----	-----	-----	-----	-----
M	L	A	I	F	W
441	450	459	468	477	486
CTA CAG ATT TTT GCC ATC CAC TCC TTA TCT GGC ATG GAA TCC ACA GTG CTG CTG					
-----	-----	-----	-----	-----	-----
L	Q	I	F	A	I
495	504	513	522	531	540
GCC ATG GCT TTT GAC CGC TAT GTG GCC ATC TGT CAC CCA CTG CGC CAT GCC ACA					
-----	-----	-----	-----	-----	-----
A	M	A	F	D	R
549	558	567	576	585	594
GTA CTT ACG TTG CCT CGT GTC ACC AAA ATT GGT GTG GCT GCT GTG GTG CGG GGG					
-----	-----	-----	-----	-----	-----
V	L	T	L	P	R
603	612	621	630	639	648
GCT GCA CTG ATG GCA CCC CTT CCT GTC TTC ATC AAG CAG CTG CCC TTC TGC CGC					
-----	-----	-----	-----	-----	-----
A	A	L	M	A	P

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FIG. 2B

657	666	675	684	693	702												
TCC	AAT	ATC	CTT	TCC	CAT	TCC	TAC	TGC	CTA	CAC	CAA	GAT	GTC	ATG	AAG	CTG	GCC
S	N	I	L	S	H	S	Y	C	L	H	Q	D	V	M	K	L	A
711	720	729	738	747	756												
TGT	GAT	GAT	ATC	CGG	GTC	AAT	GTC	GTC	TAT	GGC	CTT	ATC	GTC	ATC	ATC	TCC	GCC
C	D	D	I	R	V	N	V	V	Y	G	L	I	V	I	I	S	A
765	774	783	792	801	810												
ATT	GGC	CTG	GAC	TCA	CTT	CTC	ATC	TCC	TTC	TCA	TAT	CTG	CTT	ATT	CTT	AAG	ACT
I	G	L	D	S	L	L	I	S	F	S	Y	L	L	I	L	K	T
819	828	837	846	855	864												
GTG	TTG	GGC	TTG	ACA	CGT	GAA	GCC	CAG	GCC	AAG	GCA	TTT	GGC	ACT	TGC	GTC	TCT
V	L	G	L	T	R	E	A	Q	A	K	A	F	G	T	C	V	S
873	882	891	900	909	918												
CAT	GTG	TGT	GCT	GTG	TTC	ATA	TTC	TAT	GTA	CCT	TTC	ATT	GGA	TTG	TCC	ATG	GTC
H	V	C	A	V	F	I	F	Y	V	P	F	I	G	L	S	M	V
927	936	945	954	963	972												
CAT	CGC	TTT	AGC	AAG	CGG	CGT	GAC	TCT	CCG	CTG	CCC	GTC	ATC	TTG	GCC	AAT	ATC
H	R	F	S	K	R	R	D	S	P	L	P	V	I	L	A	N	I
981	990	999	1008	1017	1026												
TAT	CTG	CTG	GTT	CCT	CTC	AAC	CCA	ATT	GTC	TAT	GGA	GTG	AAG	ACA	AAG		
Y	L	L	V	P	P	V	L	N	P	I	V	Y	G	V	K	T	K
1035	1044	1053	1062	1071	1080												
GAG	ATT	CGA	CAG	CGC	ATC	CTT	CGA	CTT	TTC	CAT	GTG	GCC	ACA	CAC	GCT	TCA	GAG
E	I	R	Q	R	I	L	R	L	F	H	V	A	T	H	A	S	E
1089	1098	1107	1116	1125	1134												
CCC	TAG	GTG	TCA	GTG	ATC	AAA	CTT	CTT	TTC	CAT	TCA	GAG	TCC	TCT	GAT	TCA	GAT
P	*																
1143	1152	1161	1170	1179	1188												
TTT	AAT	GTT	AAC	ATT	TTG	GAA	GAC	AGT	ATT	CAG	AAA	AAA	AAT	TTC	CTT	AAT	AAA
1197	1206	1215	1224	1233	1242												
AAA	TAC	AAC	TCA	GAT	CCT	TCA	AAT	ATG	AAA	CTG	GTT	GGG	GAA	TCT	CCA	TTT	TTT
1251	1260	1269	1278	1287	1296												
CAA	TAT	TAT	TTT	CTT	CTT	TGT	TTT	CTT	GCT	ACA	TAT	AAT	TAT	TAA	TAC	CCT	GAC
1305	1314	1323	1332	1341	1350												
TAG	GTT	GTG	GTT	GGA	GGG	TTA	TTA	CTT	TTC	ATT	TTA	CCA	TGC	AGT	CCA	AAT	CTA

FIG. 2C

1359 1368 1377 1386 1395 1404  
AAC TGC TTC TAC TGA TGG TTT ACA GCA TTC TGA GAT AAG AAT GGT ACA TCT AGA  
-----  
1413 1422 1431 1440 1449 1458  
GAA CAT TTG CCA AAG GCC TAA GCA CGG CAA AGG AAA ATA AAC ACA GAA TAT AAT  
-----  
1467 1476 1485 1494 1503 1512  
AAA ATG AGA TAA TCT AGC TTA AAA CTA TAA CTT CCT CTT CAG AAC TCC CAA CCA  
-----  
1521 1530 1539 1548 1557 1566  
CAT TGG ATC TCA GAA AAA TGC TGT CTT CAA AAT GAC TTC TAC AGA GAA GAA ATA  
-----  
1575 1584 1593 1602 1611 1620  
ATT TTT CCT CTG GAC ACT AGC ACT TAA GGG GAA GAT TGG AAG TAA AGC CTT GAA  
-----  
1629 1638 1647 1656 1665 1674  
AAG AGT ACA TTT ACC TAC GTT AAT GAA AGT TGA CAC ACT GTT CTG AGA GTT TTC  
-----  
1683 1692 1701 1710 1719 1728  
ACA GCA TAT GGA CCC TGT TTT TCC TAT TTA ATT TTC TTA TCA ACC CTT TAA TTA  
-----  
1737 1746 1755 1764 1773 1782  
GGC AAA GAT ATT ATT AGT ACC CTC ATT GTA GCC ATG GGA AAA TTG ATG TTC AGT  
-----  
1791 1800 1809 1818 1827 1836  
GGG GAT CAG TGA ATT AAA TGG GGT CAT ACA AGT ATA AAA ATT AAA AAA AAA AAA  
-----  
1845 1854 1863 1872 1881 1890  
GAC TTC ATG CCC AAT CTC ATA TGA TGT GGA AGA ACT GTT AGA GAG ACC AAC AGG  
-----  
1899 1908 1917 1926 1935 1944  
GTA GTG GGT TAG AGA TTT CCA GAG TCT TAC ATT TTC TAG AGG AGG TAT TTA ATT  
-----  
1953 1962 1971 1980 1989 1998  
TCT TCT CAC TCA TCC AGT GTT GTA TTT AGG AAT TTC CTG GCA ACA GAA CTC ATG  
-----  
2007 2016 2025 2034 2043 2052  
GCT TTA ATC CCA CTA GCT ATT GCT TAT TGT CCT GGT CCA ATT GCC AAT TAC CTG  
-----  
2061 2070 2079 2088 2097 2106  
TGT CTT GGA AGA AGT GAT TTC TAG GTT CAC CAT TAT GGA AGA TTC TTA TTC AGA  
-----  
2115 2124 2133 2142 2151 2160  
AAG TCT GCA TAG GGC TTA TAG CAA GTT ATT TAT TTT TAA AAG TTC CAT AGG TGA  
-----  
2169 2178 2187 2196 2205 2214  
TTC TGA TAG GCA GTG AGG TTA GGG AGC CAC CAG TTA TGA TGG GAA GTA TGG AAT  
-----  
2223 2232 2241 2250 2259 2268  
GGC AGG TCT TGA AGA TAA CAT TGG CCT TTT GAG TGT GAC TCG TAG CTG GAA AGT  
-----  
2277 2286 2295 2304 2313 2322  
GAG GGA ATC TTC AGG ACC ATG CTT TAT TTG GGG CTT TGT GCA GTA TGG AAC AGG  
-----  
2331 2340 2349 2358 2367 2376  
GAC TTT GAG ACC AGG AAA GCA ATC TGA CTT AGG CAT GGG AAT CAG GCA TTT TTG

FIG. 2D

2385	2394	2403	2412	2421	2430
CTT	CTG	AGG	GGC	TAT	TAC
CAA	CAG	TGT	TAA	CCA	AGA
ATG	TGG	TAA	GTT	TCA	TTT
TAT	AAC	ATG	CTT	TCA	TCC
TTA	ATA	CTT	GTA	TTT	GCT
TGA	ATG	TCA	TCT	CTG	TTC
AGC	AAA	GTG	CCT	AGA	ACA
2439	2448	2457	2466	2475	2484
CAA	CAG	TGT	TAA	CCA	AGA
ATG	TGG	TAA	GTT	TCA	TTT
TAT	AAC	ATG	CTT	TCA	TCC
TTA	ATA	CTT	GTA	TTT	GCT
TGA	ATG	TCA	TCT	CTG	TTC
AGC	AAA	GTG	CCT	AGA	ACA
2493	2502	2511	2520	2529	2538
2547	2556	2565	2574	2583	2592
TAT	AAC	ATG	CTT	TCA	TCC
TTA	ATA	CTT	GTA	TTT	GCT
TGA	ATG	TCA	TCT	CTG	TTC
AGC	AAA	GTG	CCT	AGA	ACA
2601	2610	2619	2628	2637	2646
2655	2664	2673	2682	2691	2700
2709	2718	2727	2736	2745	2754
2763	2772	2781	2790	2799	2808
2817	2826	2835	2844	2853	2862
2871	2880	2889	2898	2907	2916
2925	2934	2943	2952	2961	2970
2979	2988	2997	3006	3015	3024
3033	3042	3051	3060	3069	3078
3087	3096	3105	3114	3123	3132
AAA A 3'					

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Figure 3: Protein Sequence for 101P3A11.

MVDPNGNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDILI  
STSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLRHATVTLPRVTKIGV  
AAVVRGAALMAPLPFIKQLPFCRSNILSHSYCLHQDVMKLACDDIRVNVYGLIVIISAIGLDSLLISFSYL  
LILKTVLGLTREAQAKAFGTCVSHVCAVFIFYVPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLPNPIVYG  
VKTKEIRQRILRLFHVATHASEP

Figure 4

Alignment of 101P3A11 (Sbjct) with mouse olfactory receptor S25 (Query)

Query: 34 GNYTVVTEFILLGLTDDITVSILFVFMFLIVYSVTLGNLNIIIVLIRTSPQLHTPMYLFL 93  
GN + T FIL+GL L +Y + ++GNL II ++RT LH PMY+FL

Sbjct: 6 GNESSATYFILIGLPGLEEAQFWLAFPLCSLYLIAVGLNLIIYIVRTEHSLHEPMYIFL 65

Query: 94 SHLAFLDIGYSSSVTPIMLRGFLRKGTFIPVAGCVAQLCIVVAFGTSESFLLASMAYDRY 153  
L+ +DI S+S P ML F T I C+ Q+ + + ES +L +MA+DRY

Sbjct: 66 CMLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRY 125

Query: 154 VAICSPLLYSTQMSSTVCILLVGTSYLGGWVNAWIFTGCSLNLSFCGPNKINHFFCDYSP 213  
VAIC PL ++T ++ + + + G L FC N ++H +C +

Sbjct: 126 VAICHPLRHATVLTLPRTKIGVAAVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQD 185

Query: 214 LLKLSCSHDFSFEVIPAISSGSIIIVVTVFIIIALSYVYILVSILKMRSTEGRQKAFSTCTS 273  
++KL+C V I S I + +I+ SY+ IL ++L + + E + KAF TC S

Sbjct: 186 VMKLACDDIRVNYYGLIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVS 244

Query: 274 HLTAVTLFFGTITFIYVMPQSSYSTDQNK---VVSVFYTVVIPMLNPLIYSFRNKEVKE 329  
H+ AV +F+ + FI + +S ++ +++ Y +V P+LNP++Y + KE+++

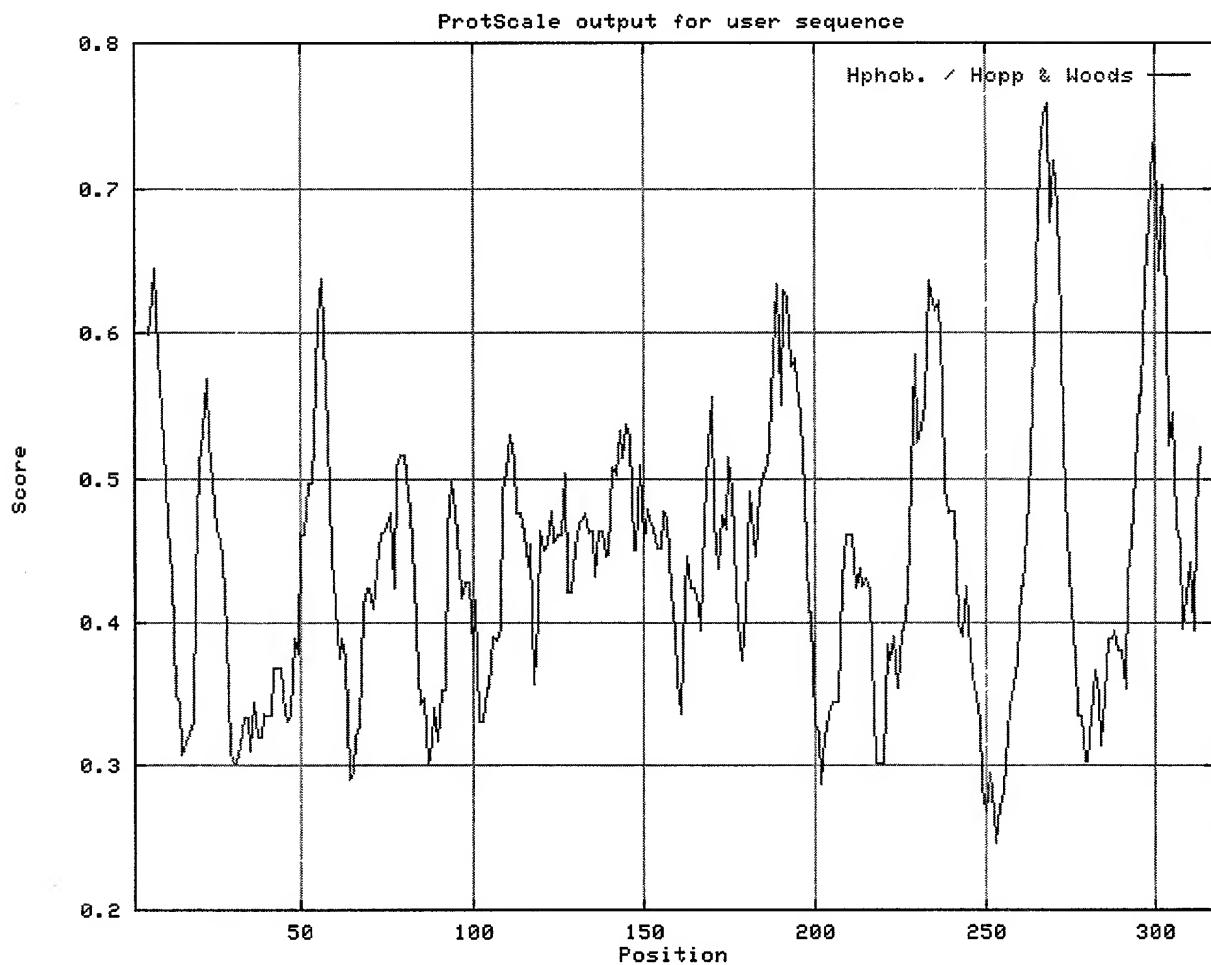
Sbjct: 245 HVCASFIFY--VPFIGLSMVHRSKRRDPLPVILANIYLLVPPVLPNIVYGVKTKEIRQ 302

Query: 330 AMKKL 334

+ +L

Sbjct: 303 RILRL 307

Figure 5:  
101P3A11 Hydrophilicity profile  
(Hopp T.P., Woods K.R., 1981. Proc. Natl. Acad. Sci. U.S.A. 78:3824-3828)



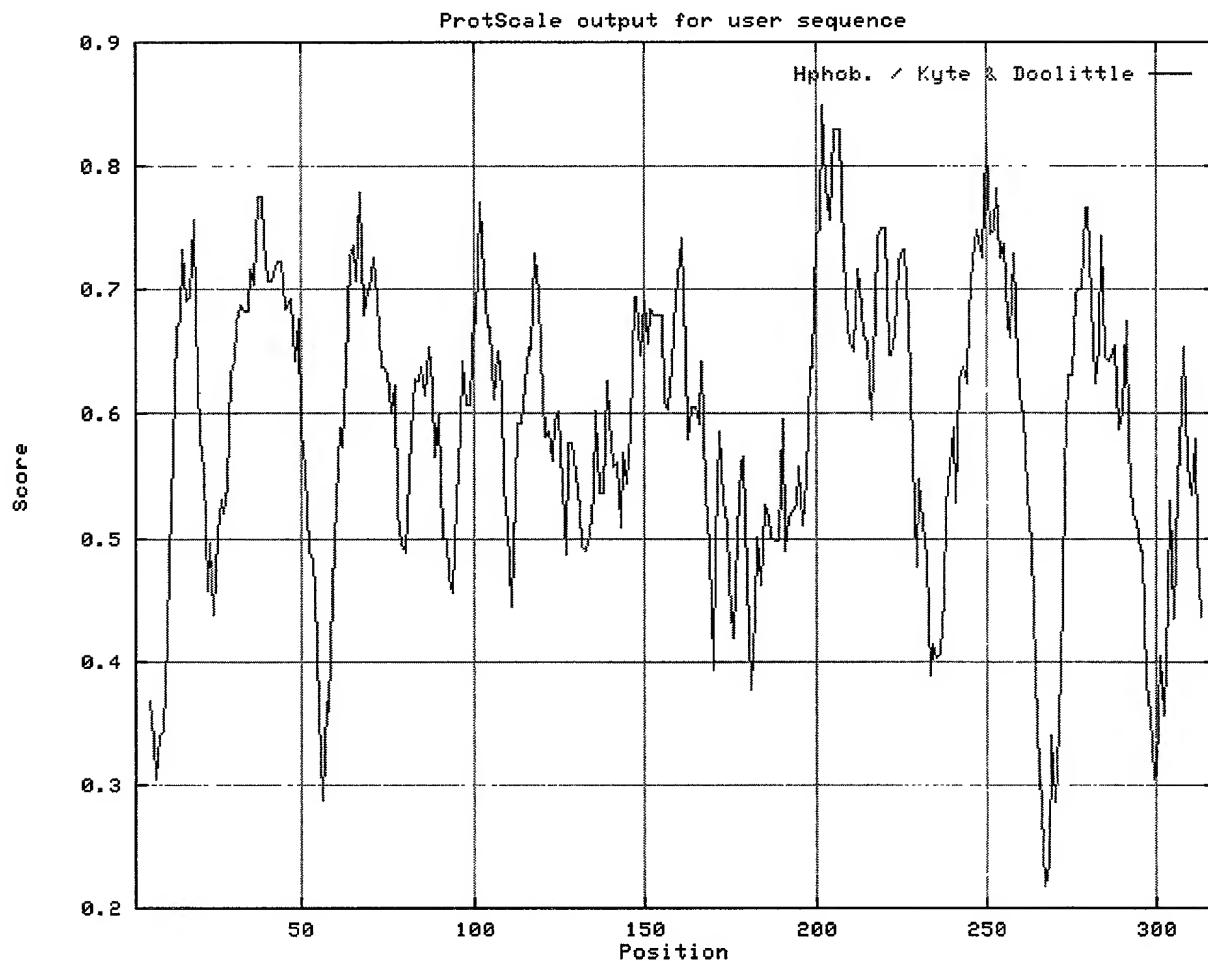
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Figure 6:  
101P3A11 Hydropathicity Profile  
(Kyte J., Doolittle R.F., 1982. J. Mol. Biol. 157:105-132)



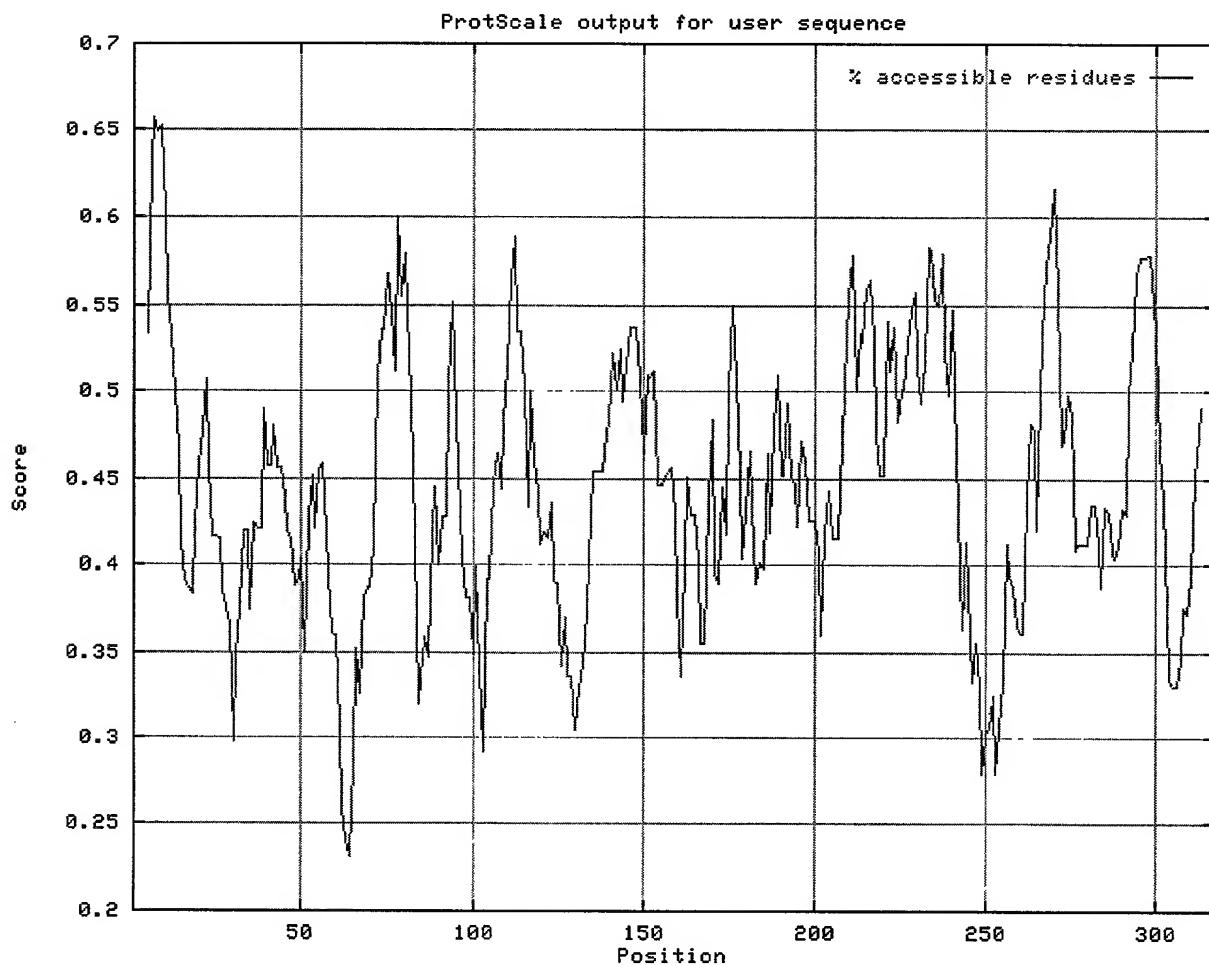
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Figure 7:  
101P3A11 % Accessible Residues Profile  
(Janin J., 1979. Nature 277:491-492)



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Figure 8:  
101P3A11 Average Flexibility Profile  
(Bhaskaran R., Ponnuswamy P.K., 1988.  
Int. J. Pept. Protein Res. 32:242-255)

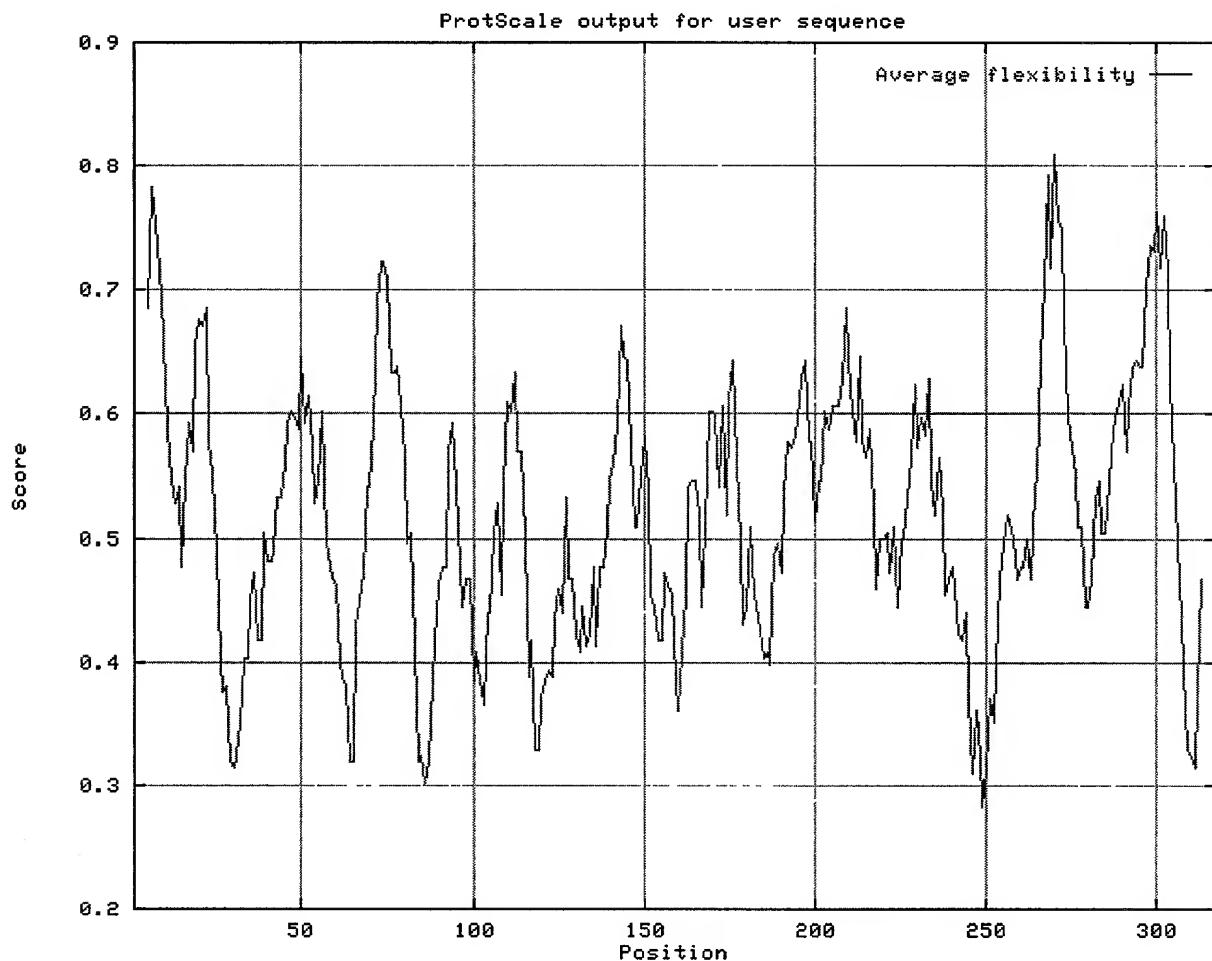
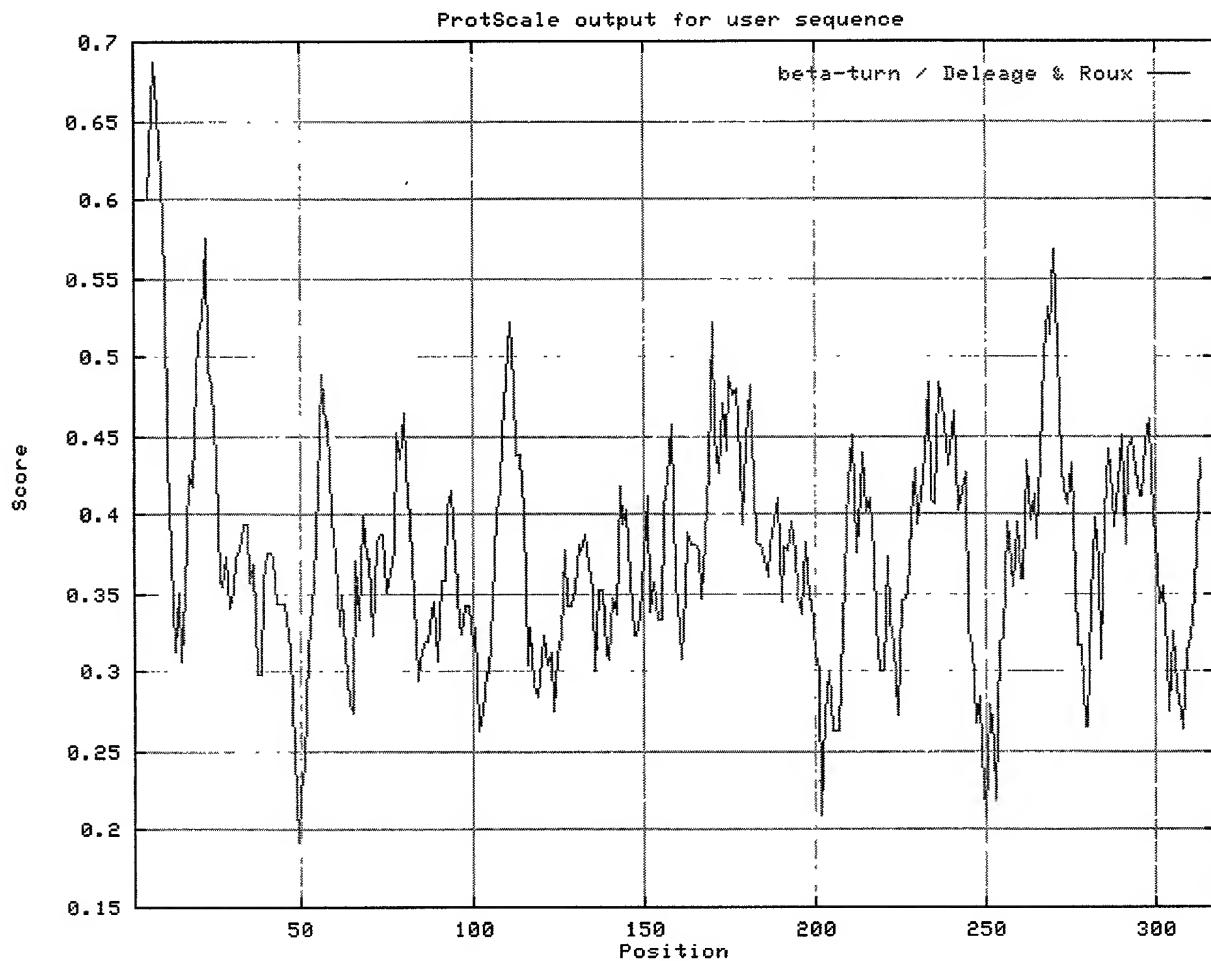
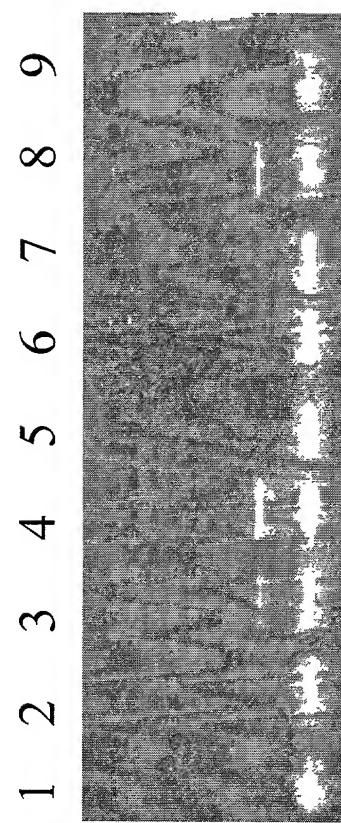


Figure 9:  
101P3A11 Beta-turn Profile  
(Deleage, G., Roux B. 1987. Protein Engineering 1:289-294)

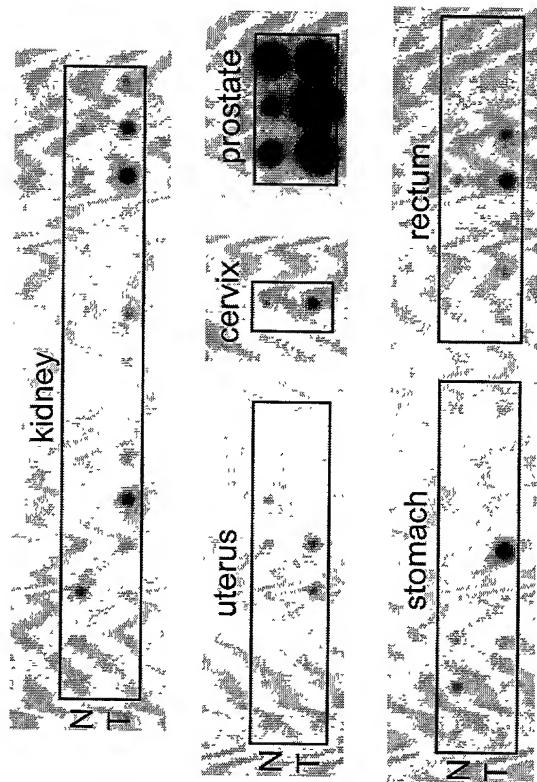


## Figure 10A. Expression of 101P3A11 by RT-PCR



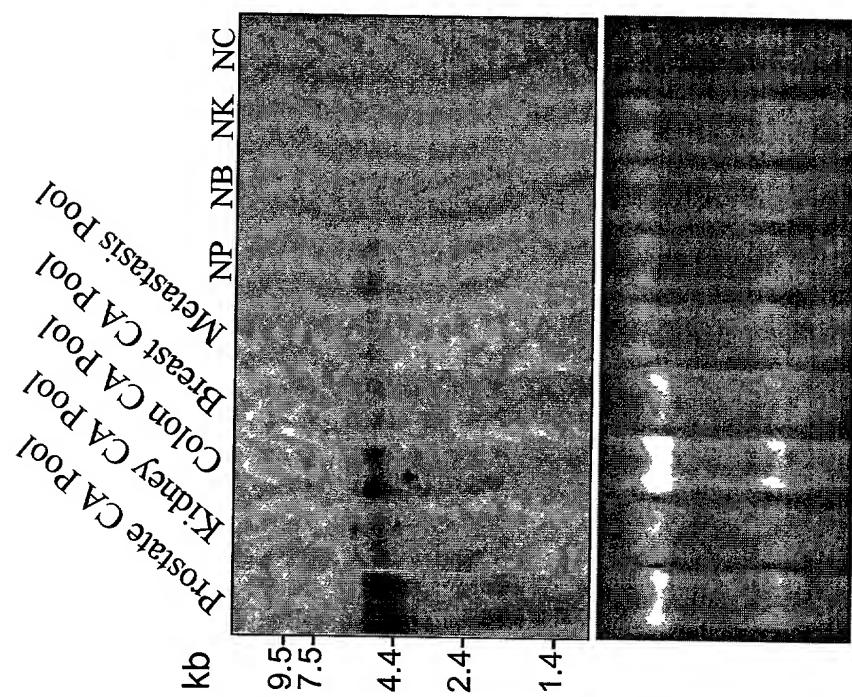
- VP1 (Kidney, Lung, Liver)
- VP2 (Pancreas, Colon, Stomach)
- Prostate xenograft Pool
- Prostate Cancer Pool
- Kidney Cancer Pool
- Colon Cancer Pool
- Breast Cancer Pool
- Metastasis Pool
- H2O

**Figure 10B**



**Figure 11. Expression of 101P3A11 in Human Patient Cancer Specimens**

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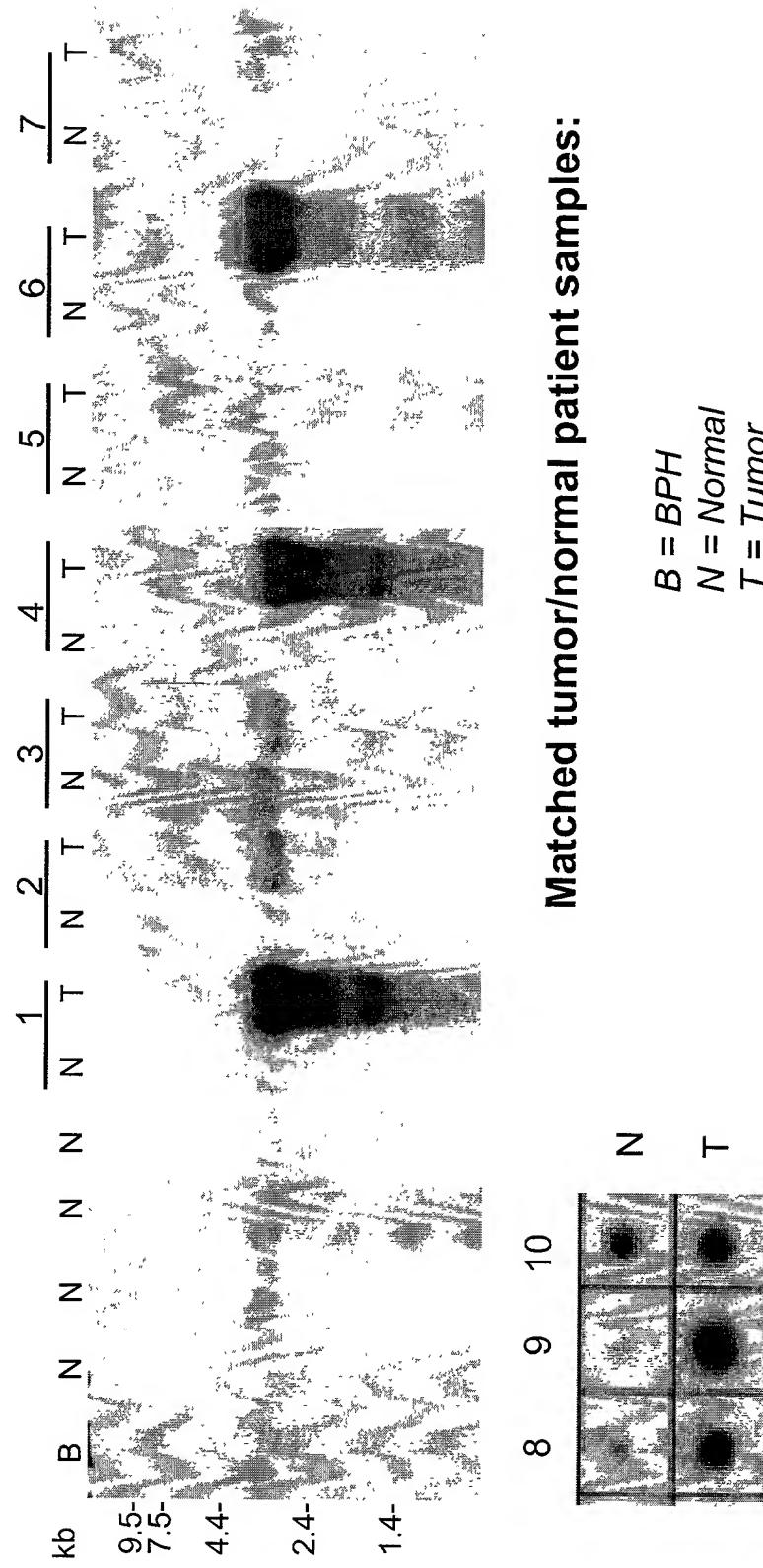


10 $\mu$ g total RNA/per lane from a pool of 3 tumors as follows:

Prostate Cancer Pool = gleason 6, 8, 9  
Kidney Cancer Pool = grade 2, 2, 3  
Colon Cancer Pool = stage II, III, IV  
Breast Cancer Pool = grade 1, 2, 3  
Metastasis Pool = colon to lung, colon to liver, ovary to fall. tube

NP = Normal Prostate  
NB = Normal Bladder  
NK = Normal Kidney  
NC = Normal Colon

# Figure 12A



## Figure 12B and 12C

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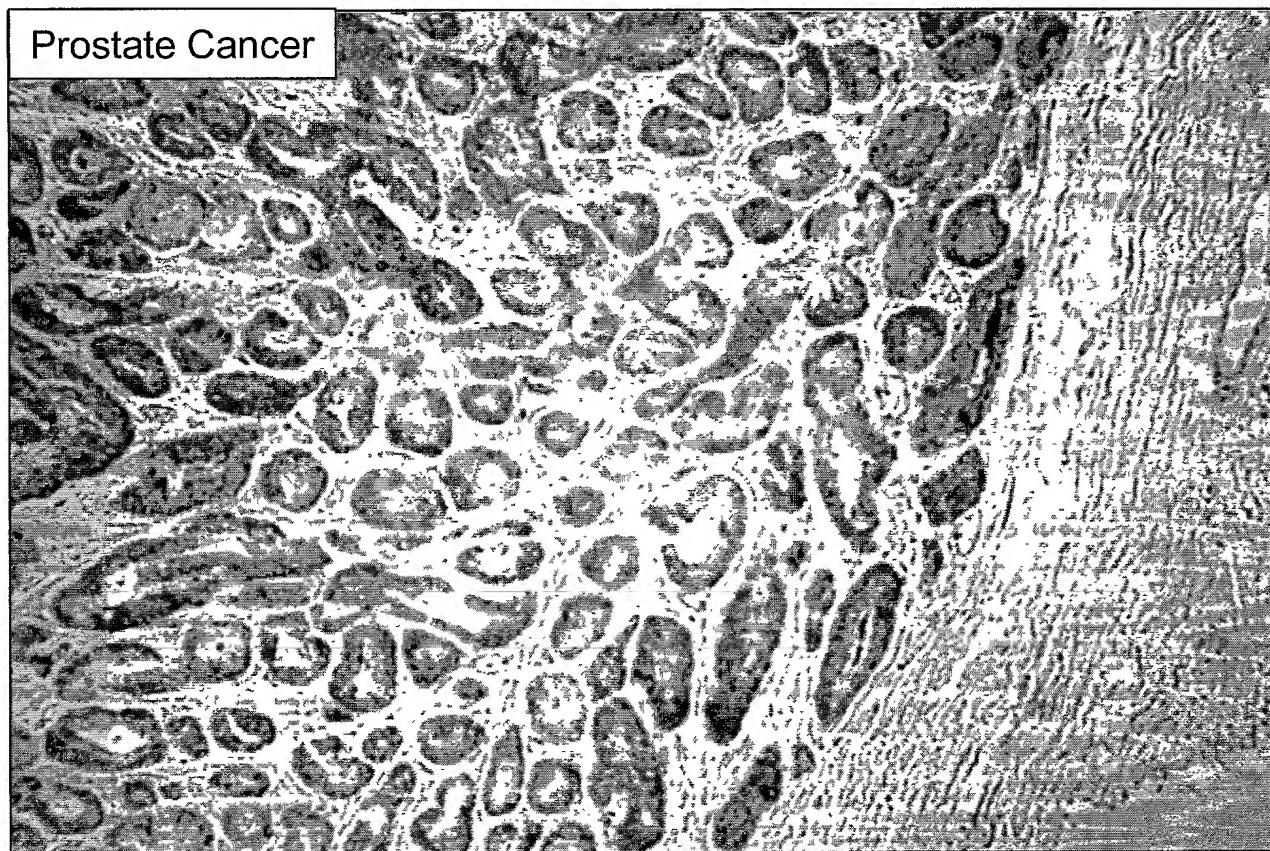
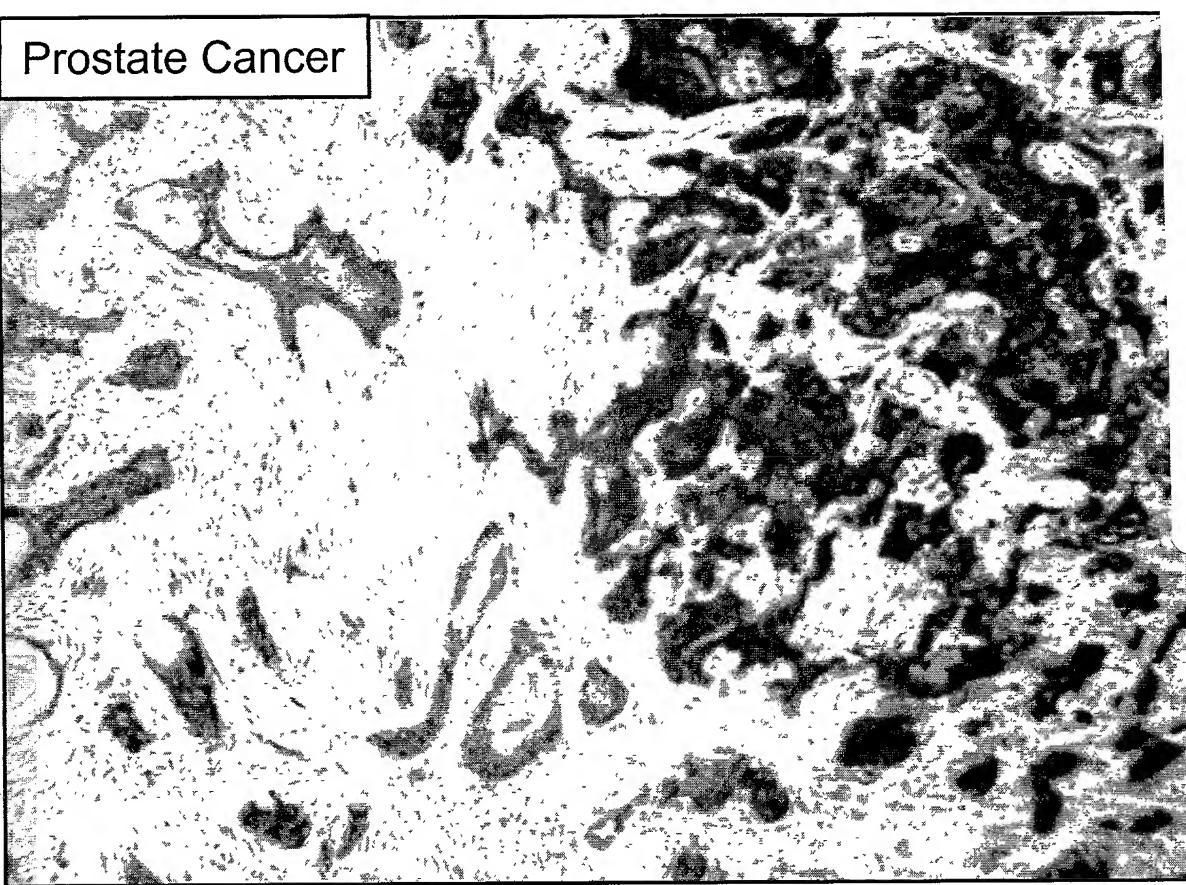
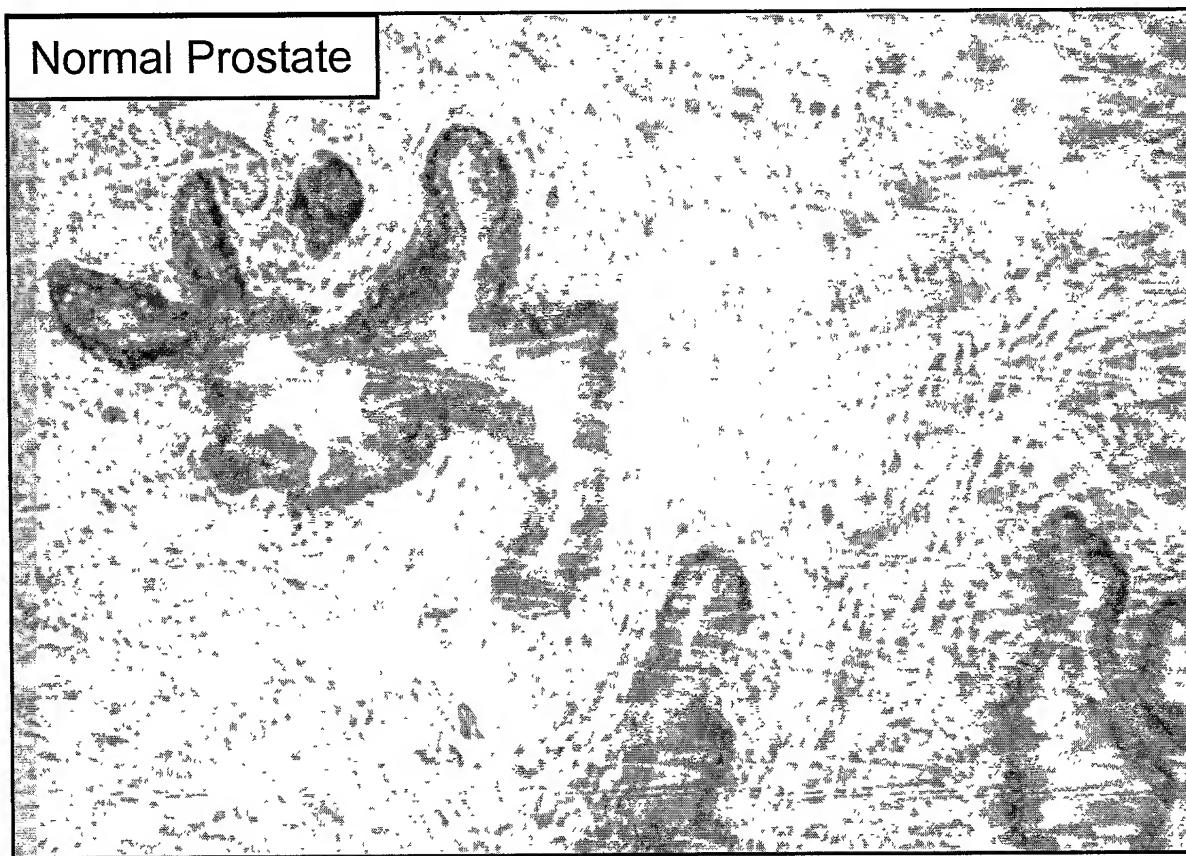


Figure 12D and 12E

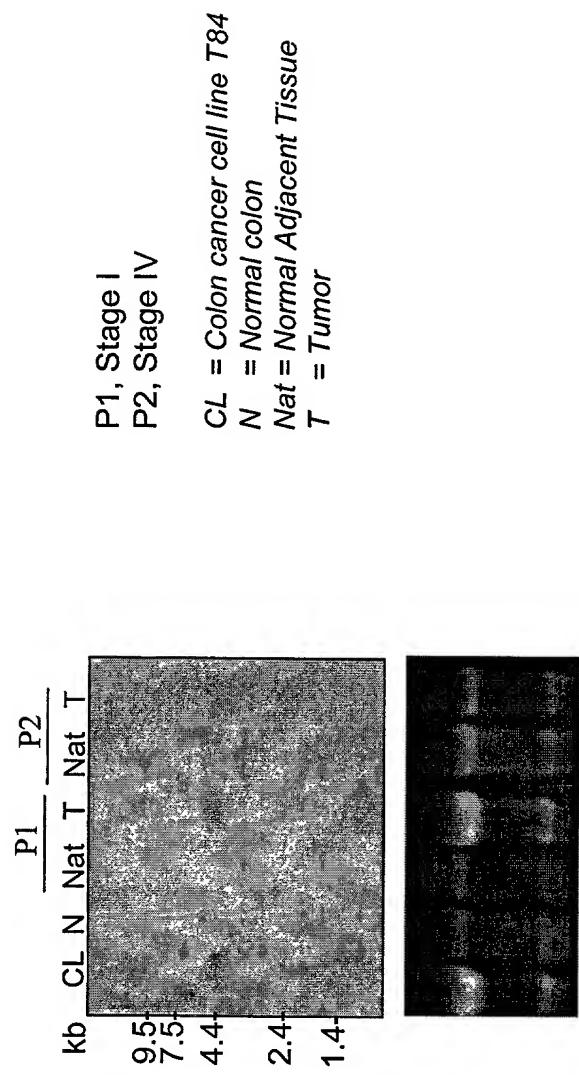
Prostate Cancer



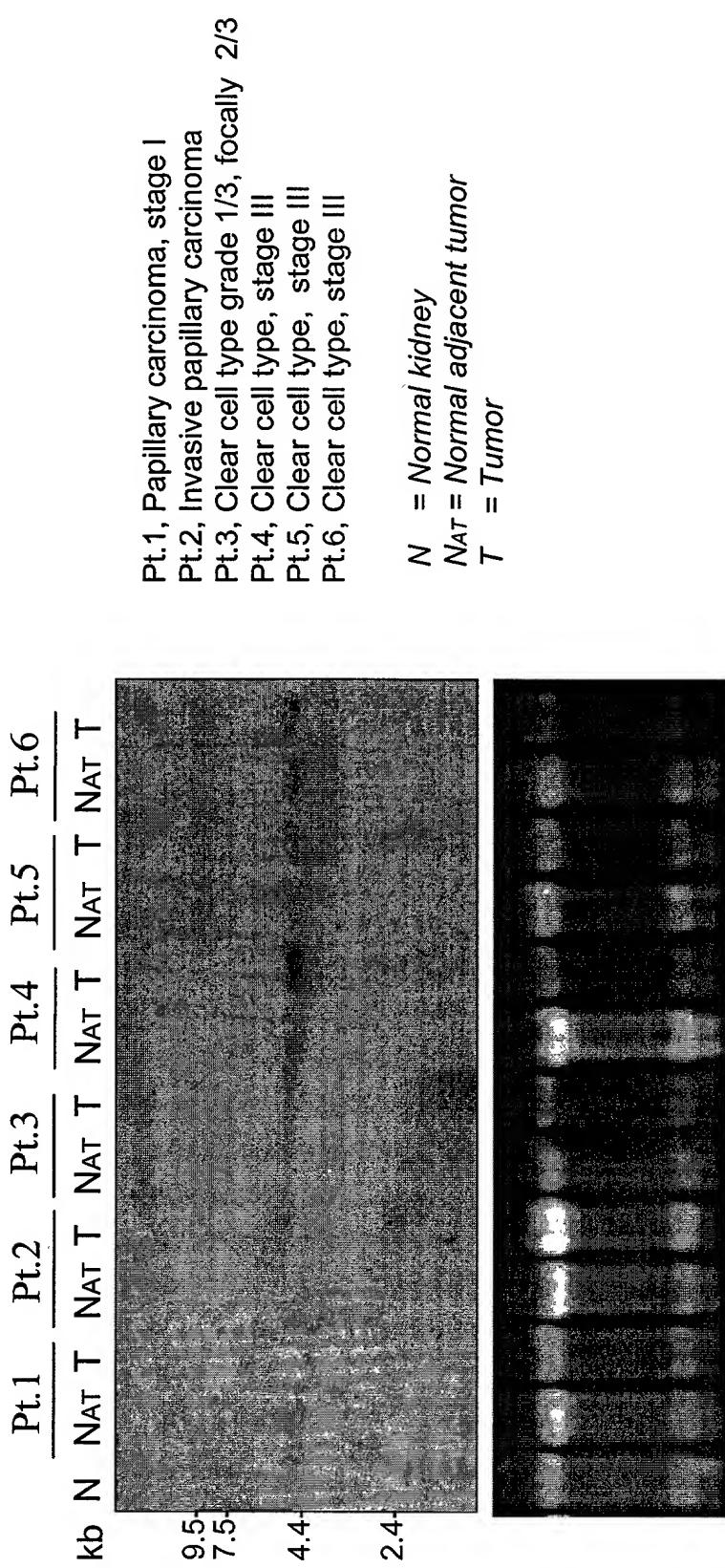
Normal Prostate



**Figure 13. Expression of 101P3A11 in Colon Cancer Patient Specimens**

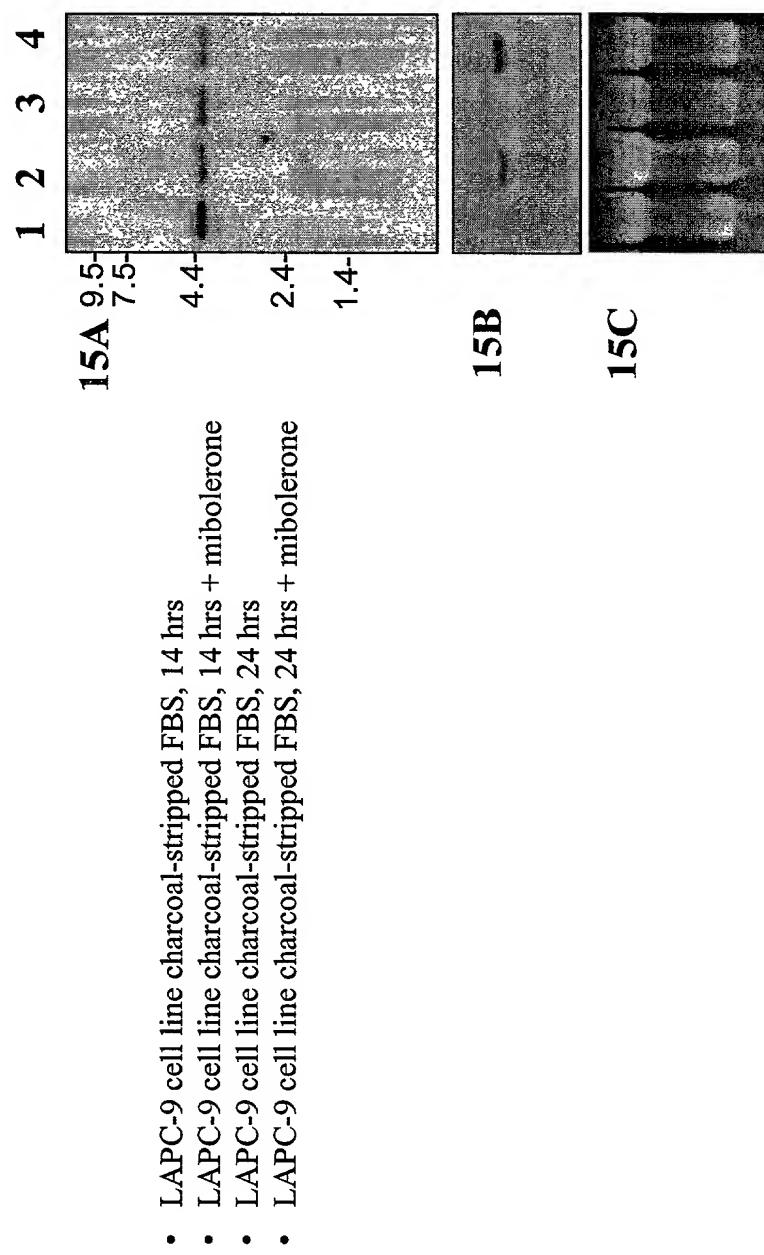


**Figure 14. Expression of 101P3A11 in Kidney Cancer Patient Specimens**



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Figure 15A-15C. Androgen Regulation of 101P3A11 in Tissue Culture Cells



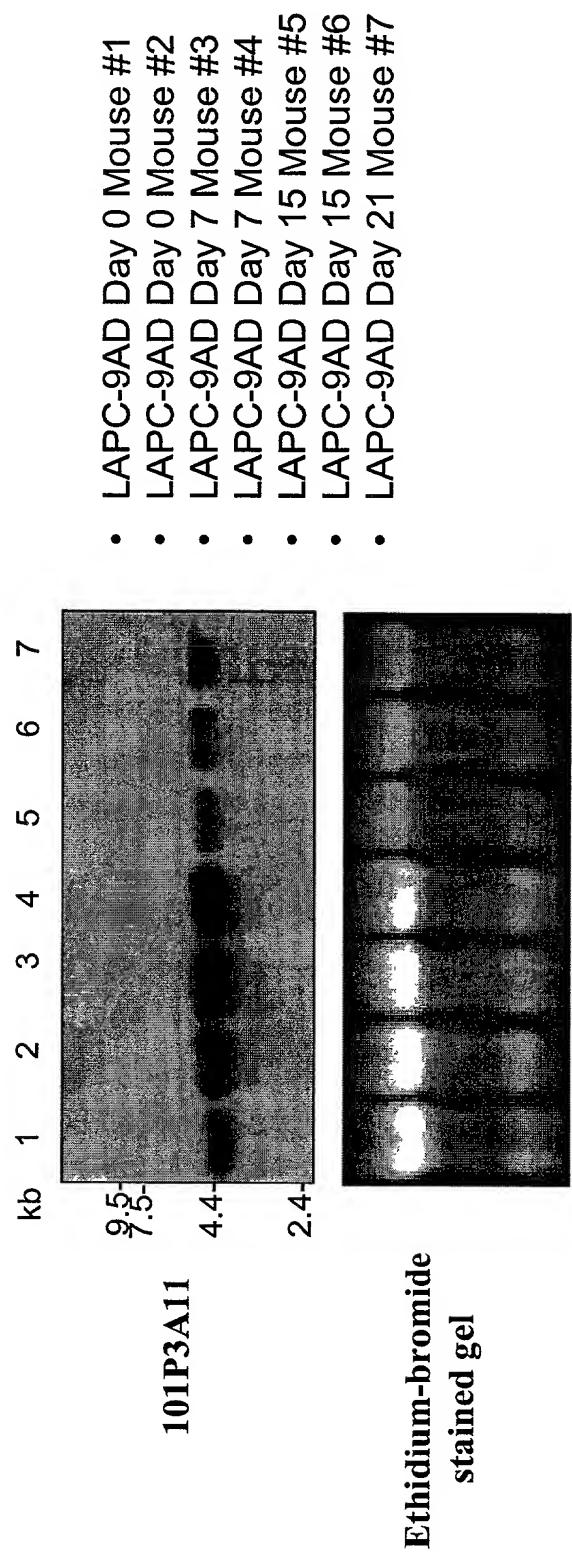
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**Figure 16. Androgen Regulation of 101P3A11 *In Vivo***



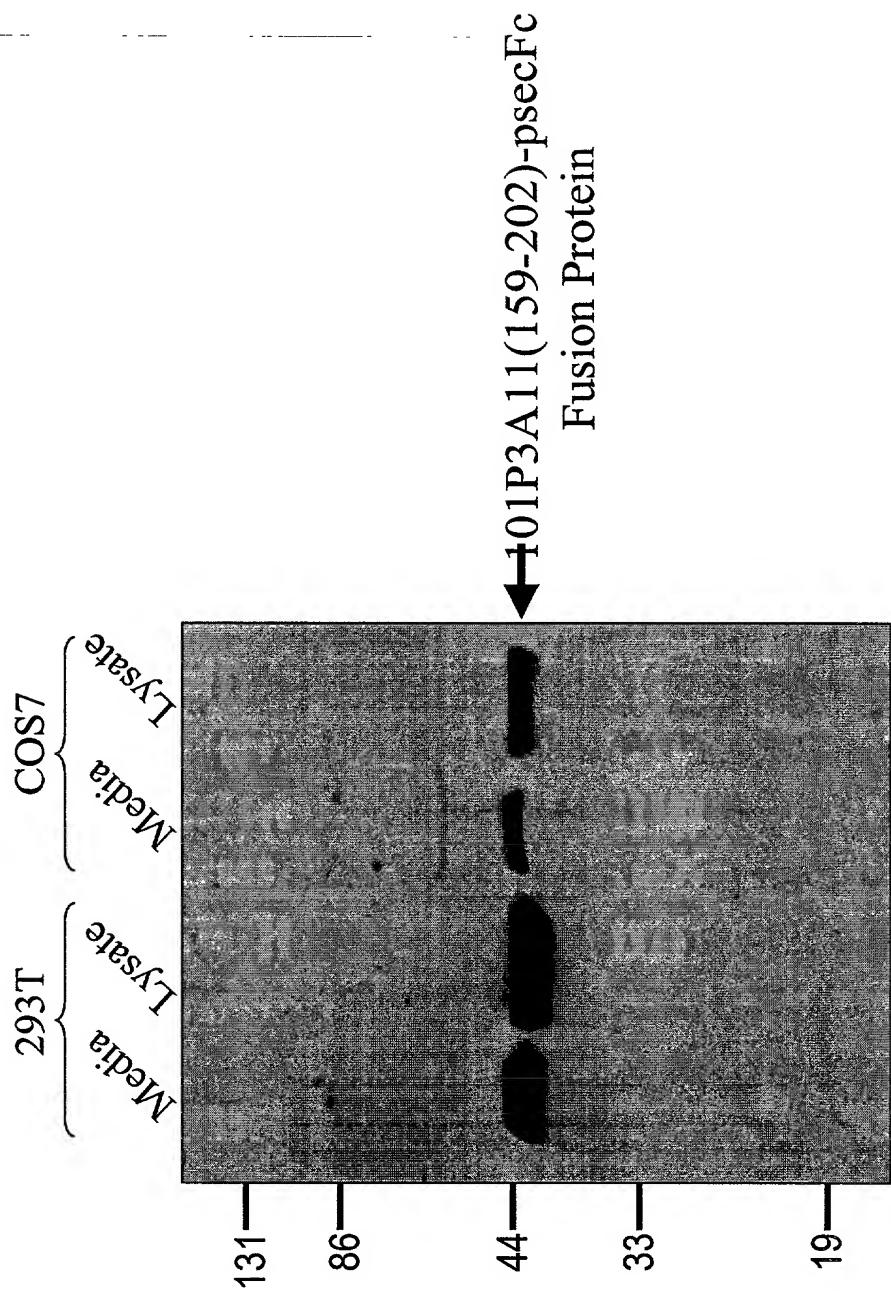
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**Figure 17. Expression and Detection of 101P3A11(159-202)-psecFc Fusion Protein**



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**Figure 18. Expression of 101P3A11 in 300.19 Cells**

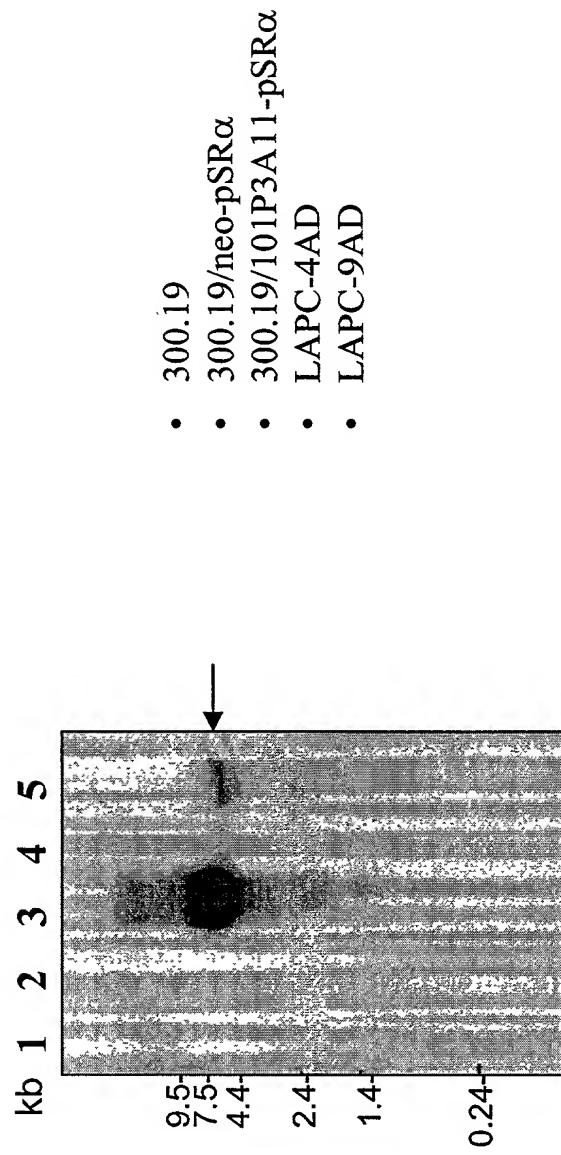
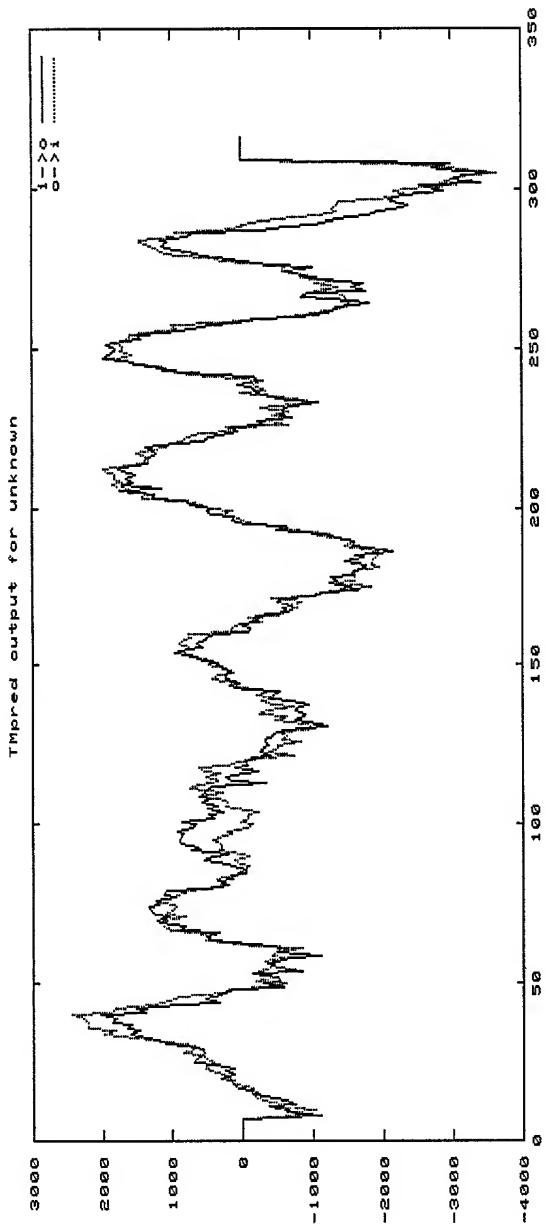


Figure 19A. Secondary structure prediction of 101P3A11

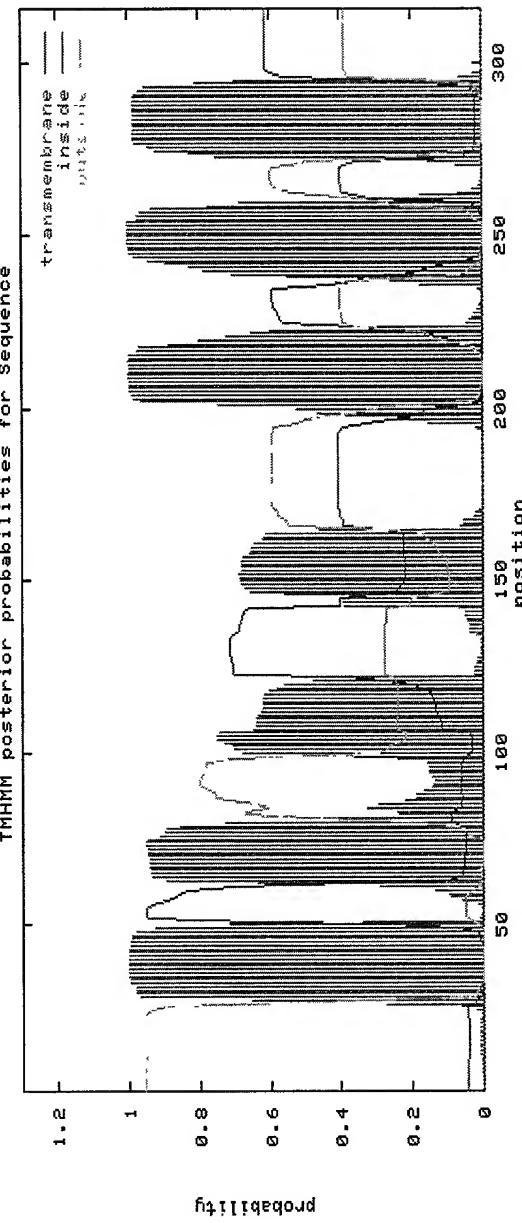
c: random coil (30.60%)  
e: extended strand (21.45%)  
h: alpha helix (47.95%)

**Figure 19B-19C. Transmembrane prediction of 101P3A11**

**19B**



**19C**



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DETECTION OF CANCER

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Figure 20. Expression of 101P3A11 in NIH-3T3 Tumors

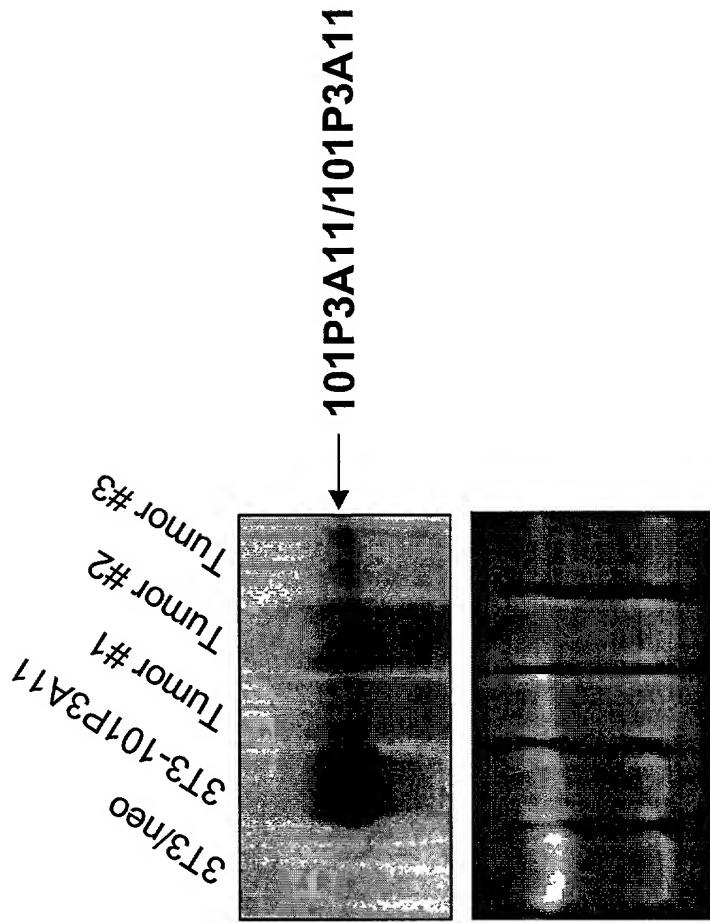
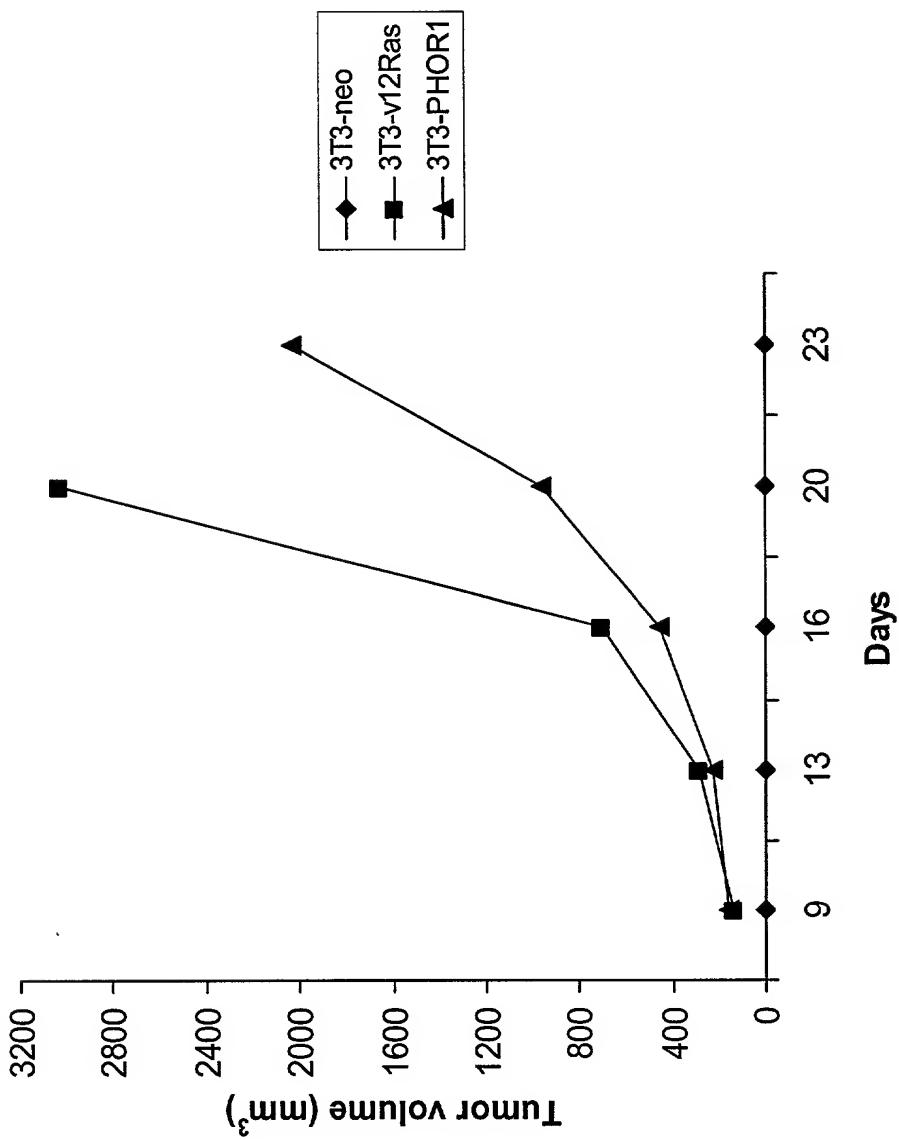
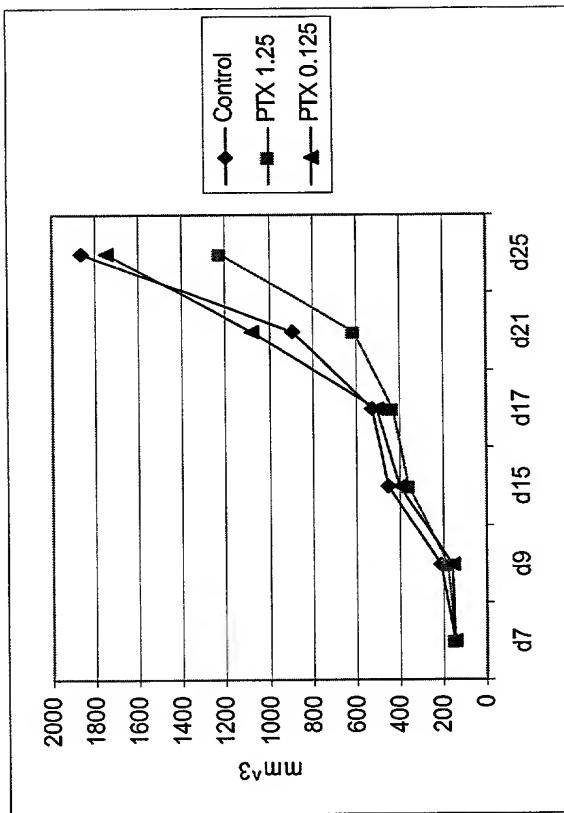


Figure 21: 101P3A11 Induces Tumor Formation of 3T3 Cells



• Injection of  $10^6$  3T3-neo, 3T3-Ras or 3T3-101P3A11 cells subcutaneously into SCID mice revealed that 6/6 3T3-Ras-injected mice formed tumors, 6/6 3T3-101P3A11- injected mice formed tumors, and 0/6 3T3-neo-injected mice formed tumors.

Figure 22: PTX Reduces the *in vivo* Growth of 3T3-101P3A11 Tumors



- Pertussis toxin inhibits the sub-cutaneous growth of 3T3-101P3A11 tumors in SCID mice.
- The inhibitory activity of pertussis toxin occurs in a dose dependent manner.

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Figure 23: Alignment of 101P3A11-PHOR-1 with the rat GPCR RA1C (gi|3420759).

Identities = 179/299 (59%), Positives = 231/299 (76%), Gaps = 1/299 (0%)

PHOR:	14	FILIGLPGLEEAQFWLAFFPLCSLYLIAVLGNLTIIYIVRTEHSIHEPMYIFLCMLSGIDI	73
		F+LIG+PGLEEA FW FPL S+Y +A+ GN +++IVRTE SLH PMY+FLCML+ ID+	
RA1C:	11	FMLIGIPGLEEAHFWFGFPLLSMYAVALFGNCIVVFIVRTERSLHAPMYLFLCMLAAIDL	70
PHOR:	74	LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLR	133
		+STS+MPK+LA+FWF+S I FDACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR	
RA1C:	71	ALSTSTMPKILALFWFDSREITFDACLAQMFFIHALSAIESTILLAMAFDRYVAICHPLR	130
PHOR:	134	HATVLTLPRTVKIGVAAVVRAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLA	193
		CD HA VL +IG+ A+VRG+ PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D	
RA1C:	131	HAAVLNNTVTQIGMVALVRGSLFFFPLPLLIKRLAFCHSNVLSHSYCVHQDVMKLA	190
PHOR:	194	IRVNVVYGLIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSHVCASFIF	252
		NVYVGL I+ +G+D + IS SY LI++ VL L ++ +AKAFGTCVSH+ V F	
RA1C:	191	TLPNVVYGLTAILLVMGVDMFISLSYFLIIRAVLQLPSKSERAKAFGTCVSHIGVVLAF	250
PHOR:	253	YVPFIGLSMVHRFSKRRDPLPVILANIYLLVPPVLPNPIVYGVKTKEIRQRILRLFHVA	311
		YVP IGLS+VHRF D + V++ ++YLL+PPV+NPI+YG KTK+IR R+L +F ++	
RA1C:	251	YVPLIGLSVVHRFGNSLDPIVHVLMGDVYLLLPPVINPIIYGAKTKQIRTRVLAMFKIS	309

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Figure 24: Alignment of 101P3A11-PHOR-1 with the human prostate specific GPCR. (gi|13540539)

Identities = 179/299 (59%), Positives = 233/299 (77%), Gaps = 1/299 (0%)

PHOR: 14 FILIGLPGLEEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLCMLSGIDI 73  
F+LIG+PGL+E FW+ FPL S+Y++A+ GN +--+IVRTE SLH PMY+FLCML+ ID+  
GPCR: 11 FVLIGIPGLEKAHFVWGFPLLSMYVVMFGNCIVVFIVRTERSLHAPMYLFLCMLAAIDL 70

PHOR: 74 LISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLSGMESTVLLAMAFDRYVAICHPLR 133  
+STS+MPK+LA+FWF+S I F+ACL Q+F IH+LS +EST+LLAMAFDRYVAICHPLR  
GPCR: 71 ALSTSTMPKILALFWFDSREISFEACLTQMFFIHALSAIESTILLAMAFDRYVAICHPLR 130

PHOR: 134 HATVLTLPRTKIGVAAVVRGAALMAPLPVFIKQLPFCRSNILSHSYCLHQDVMKLACDD 193  
HA VL +IG+ AVVRG+ PLP+ IK+L FC SN+LSHSYC+HQDVMKLA D  
GPCR: 131 HAAVLNNNTVTAQIGIVAVVRGSLFFFPLPLLIKRLAFCHSNVLSHSYCVHQDVMKLAYAD 190

PHOR: 194 IRVNVVYGLIVIISAIGLDSLLISFSYLLILKTVIGL-TREAQAKAFGTCVSHVCASF 252  
NVVYGL I+ +G+D + IS SY LI++TVL L ++ +AKAFGTCVSH+ V F  
GPCR: 191 TLPNVVYGLTAILLVMGVDMFISLSYFLIIRTVLQLPSKSERAKAFGTCVSHIGVVLAF 250

PHOR: 253 YVPFIGLSMVHRFSKRRDPLPVILANIYLLVPPVLPNPIVYGVKTKEIRQRLRLFHVA 311  
YVP IGLS+VHRF + V++ +IYLL+PPV+NPI+YG KTK+IR R+L +F ++  
GPCR: 251 YVPLIGLSVVHRFGNSLHPIVRVVMGDIYLLPPVINPIIYGAKTKQIRTRVLAMFKIS 309

Figure 25: Alignment with human olfactory receptor 5I12 (gi|14423836)

Identities = 163/304 (53%), Positives = 214/304 (69%), Gaps = 1/304 (0%)

PHOR: 7 NESSATYFILIGLPGLEAQFWLAFPLCSLYLIAVLGNLTIIYIVRTEHSLHEPMYIFLC 66  
N + +F+L G+PGL + WL+ PLC +Y +A+ GN I+ VR E SLHEPMY FL  
HOR5: 5 NVTHPAFFLLTGIPGLESSHWSLGPLCVMYAVALGGNTVILQAVRVEPSLHEPMYYFLS 64

PHOR: 67 MLSGIDILISTSSMPKMLAIFWFNSTTIQFDACLLQIFAIHSLGMESTVLLAMAFDRYV 126  
MLS D+ IS +++P +L F N+ I FDACL+Q+F IH S MES +LLAM+FDRYV  
HOR5: 65 MLSFSDVAISMATLPTVLRFCLNARNITFDACLIQMFLIHFFSMMESGILLAMSFDRYV 124

PHOR: 127 AICHPLRHATVLTPLRVTKIGVAAVVRAALMAPLPVFIKQLPFCRSNILSHSYCLHQDV 186  
AIC PLR+ATVLT + +G+ A R + PLP IK+LP CRSN+LSHSYCLH D+  
HOR5: 125 AICDPLRYATVLTTEVIAAMGLGAAARSFITLFPFLIKRLPICRSNVLSHSYCLHPDM 184

PHOR: 187 MKLACDDIRVNVVYGLIVIISAIGLDSLLISFSYLLILKTVLGL-TREAQAKAFGTCVSH 245  
M+LAC DI +N +YGL V++S G+D I SY+LIL++V+ +RE + KA TCVSH  
HOR5: 185 MRLACADISINSIYGLFVLVSTFGMDLFFIFLSYVILRSVMATASREERLKALNTCVSH 244

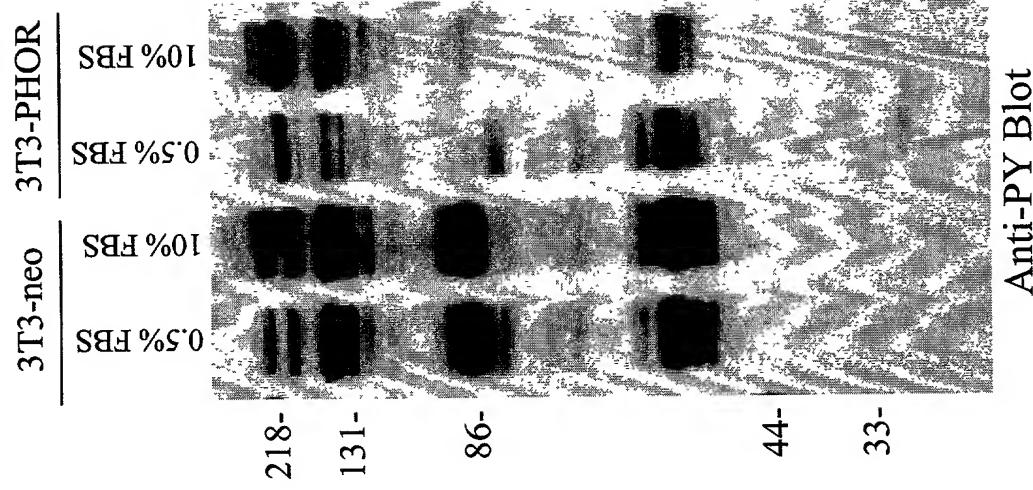
PHOR: 246 VCAVFIFYVPFIGLSMVHRFSKRRDSPLPVILANIYLLVPPVLPNPIVYGVKTKEIRQRIL 305  
+ AV FYVP IG+S VHRF K + V+++N+YL VPPVLPN+Y KTKEIR+ I  
HOR5: 245 ILAVLAFYVPMIGVSTVHRFGKHVPCYIHVLMNSVYLFVPPVLPNPLIYSAKTKEIRRAIF 304

PHOR: 306 RLFH 309

R+FH

HOR5: 305 RMFH 308

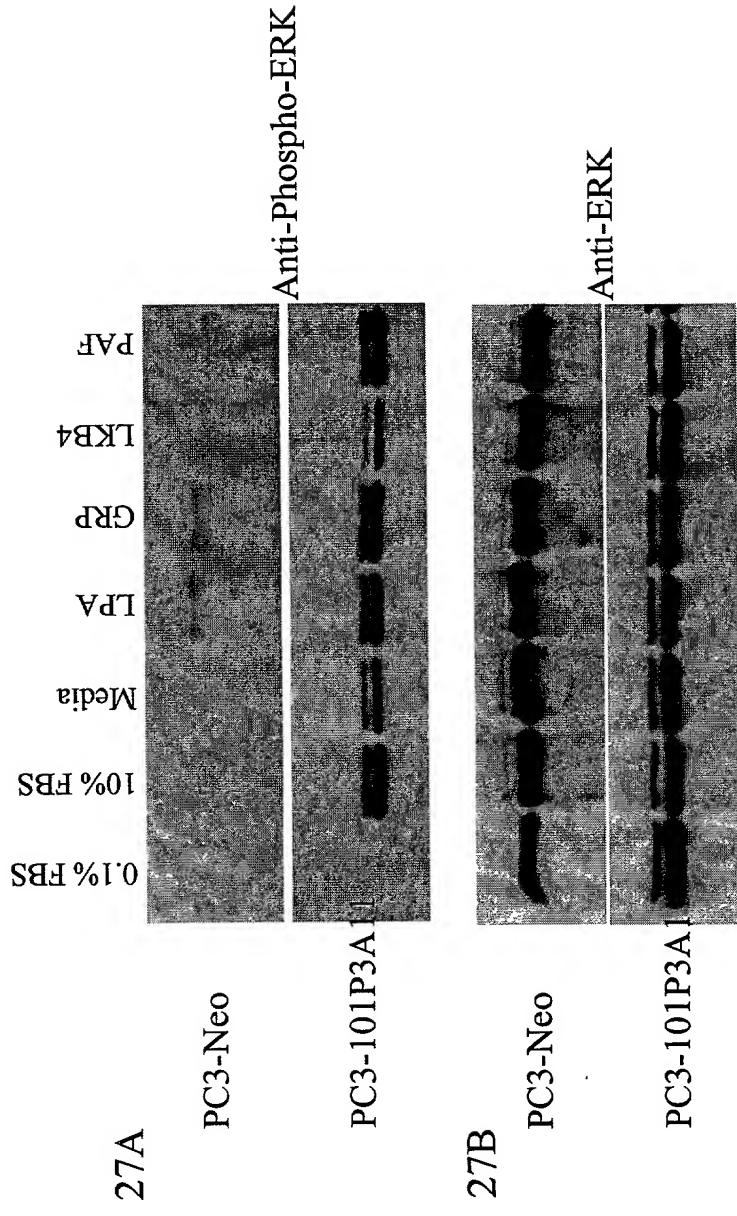
Figure 26: 101P3A11 Modulated Tyrosine Phosphorylation in NIH-3T3 Cells



Anti-PY Blot

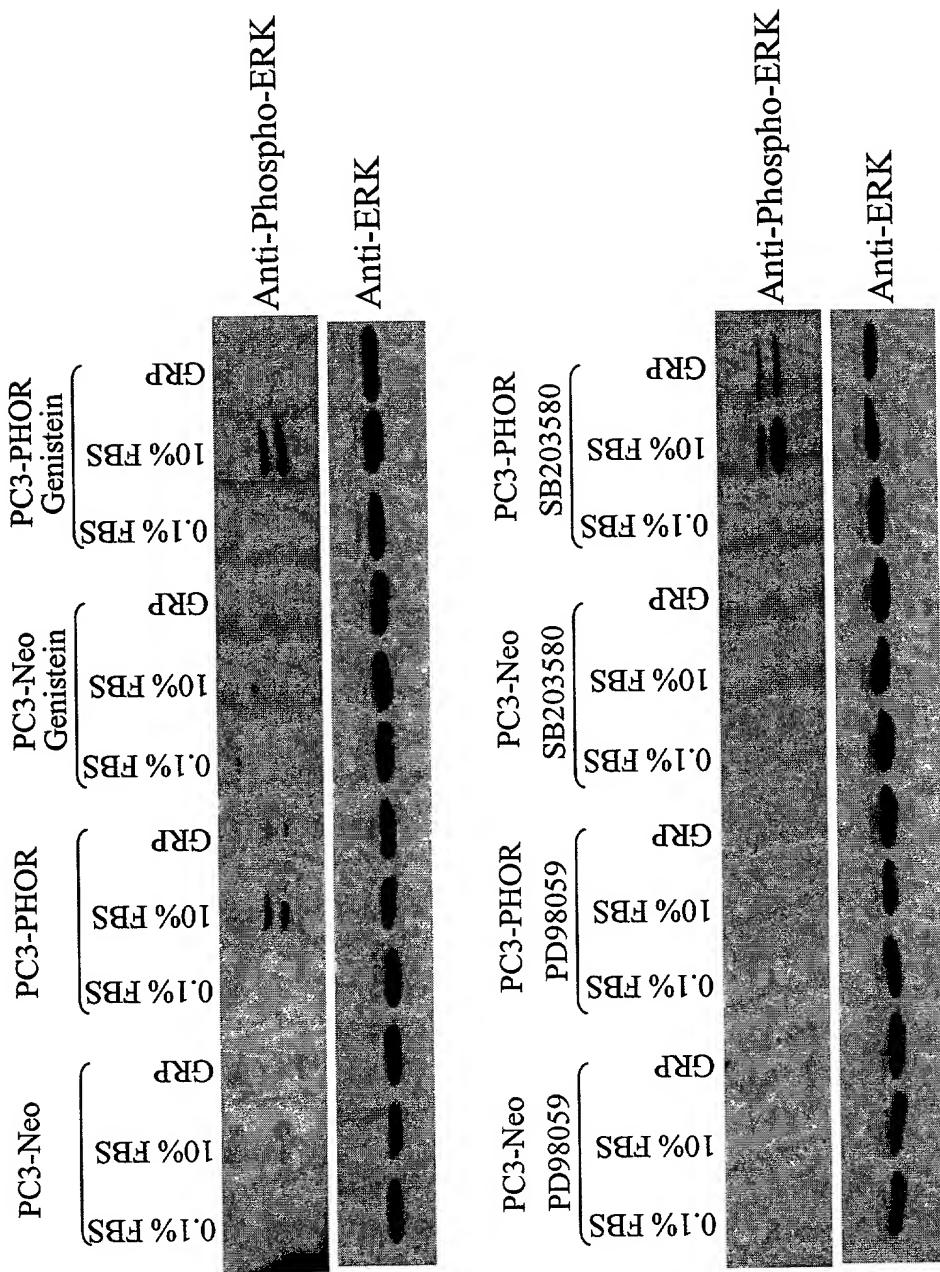
- 101P3A11 mediated the de-phosphorylation of proteins at 200, 120-140, 85-90 and 55 kDa
- 101P3A11 induced the phosphorylation of proteins at 80 and 29 kDa in NIH-3T3 cells.

## Figures 27A-27B: ERK Phosphorylation by PCR Ligands in 101P3A11 Expressing Cells



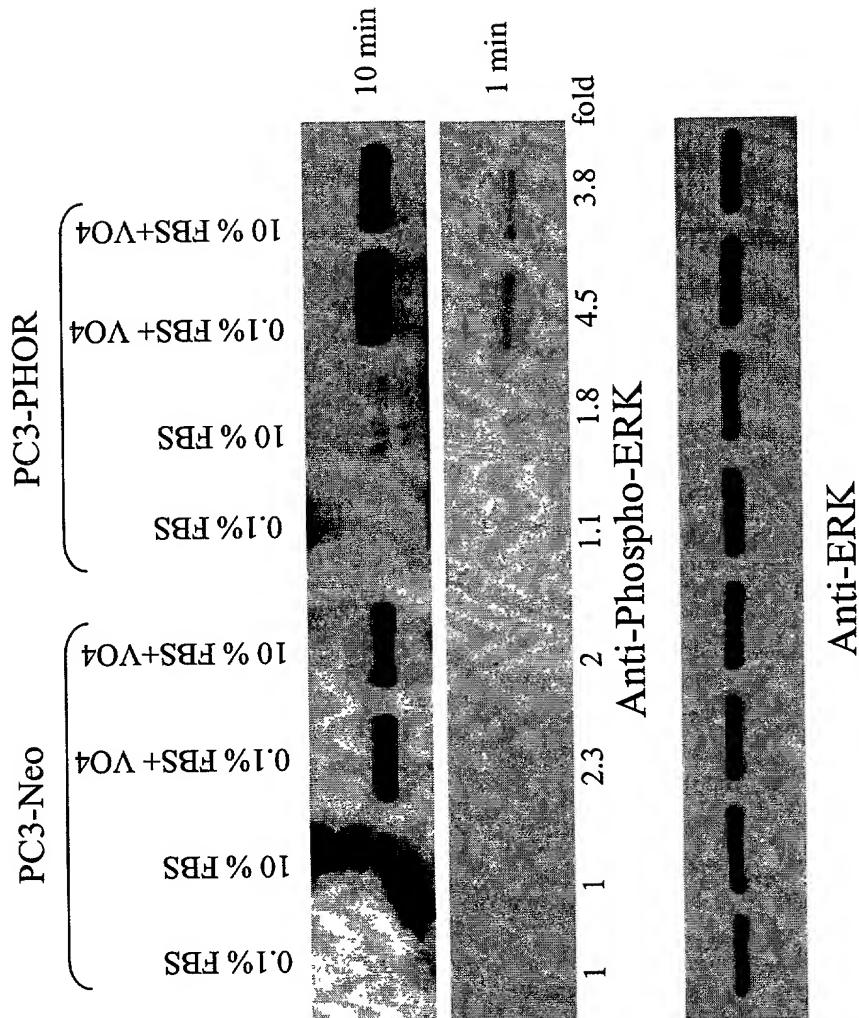
- FBS, ipophosphatidic acid, gastrin releasing peptide, leukotriene and platelet activating factor induced the phosphorylation of ERK in 101P3A11 expressing cells.

**Figure 28: Inhibition of 101P3A11-Mediated ERK Activation by PD98059**



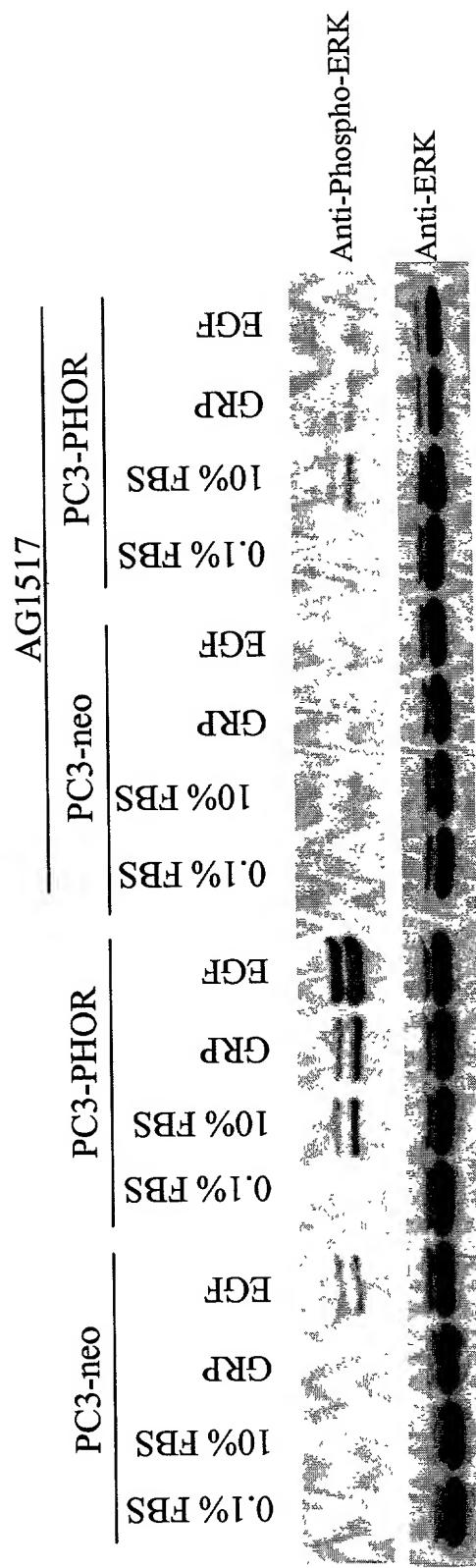
•ERK phosphorylation was inhibited by a MEK specific(PD98059) but not a p38 specific (SB203580) inhibitor in PC3-101P3A11 cells.

Figure 29: Enhanced ERK Phosphorylation in Sodium Orthovanadate Treated PC3-101P3A11 Cells



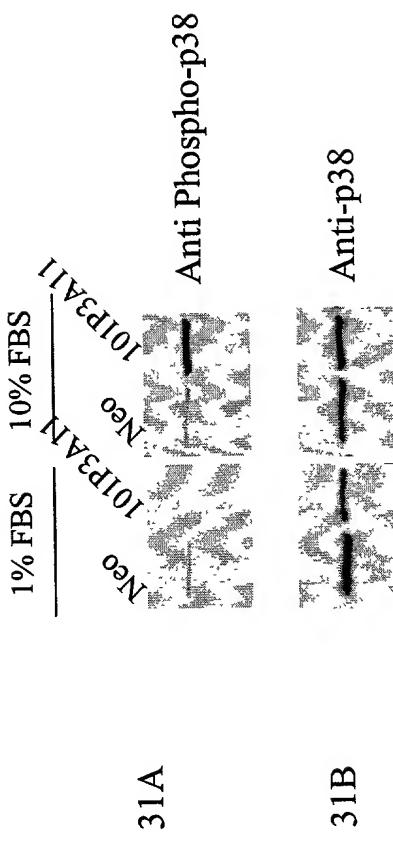
•Sodium orthovanadate induced increased ERK phosphorylation in PC3-101P3A11 cells relative to PC3-neo cells.

Figure 30: Inhibition of 101P3A11-Mediated ERK Phosphorylation by AG1517



- The EGFR inhibitor, AG1517, inhibits EGF-mediated ERK phosphorylation in control and 101P3A11 expressing PC3 cells.
- AG1517 partially inhibits 101P3A11 mediated ERK phosphorylation in PC3 cells.

Figure 31A-31B: Activation of p38 in PC3-101P3A11 Cells



•Expression of 101P3A11 mediates p38 phosphorylation in cells treated with 10% FBS.

Figure 32: 101P3A11 Induced Accumulation of cAMP in PC3 Cells

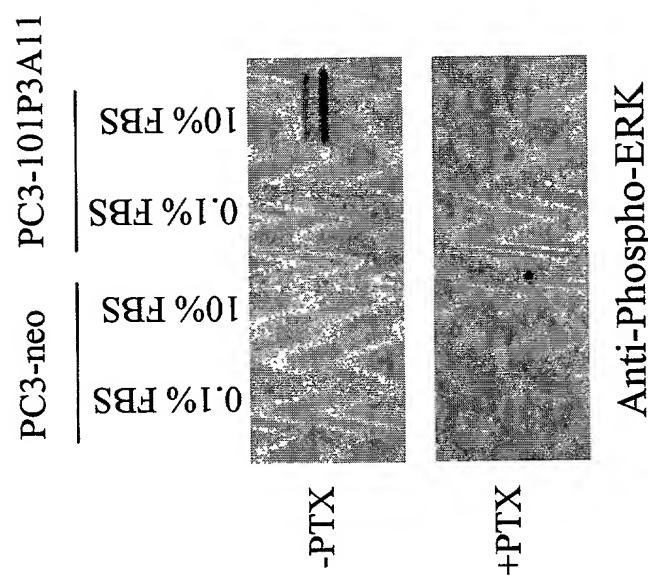
		Fold change in [cAMP]	
		PC3-Neo	PC3-PHOR
0.1%FBS	-PTX	1	4.302
	+PTX	1.403	2.577
10%FBS	-PTX	2.738	6.978
	+PTX	2.163	2.752

Fold Change in cAMP accumulation was calculated relative to PC3-neo cells grown in 0.1%FBS

•Expression of 101P3A11 increased the accumulation of cAMP in cells treated with 0.1% and 10% FBS.

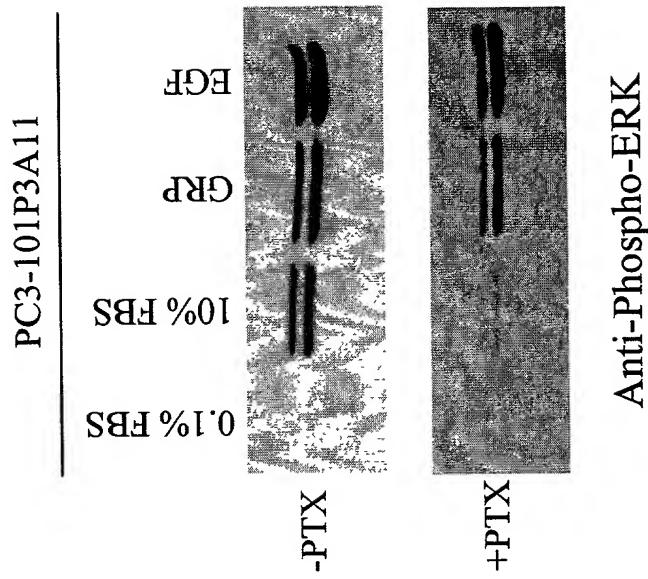
•FBS-induced cAMP accumulation in 101P3A11 cells was inhibited by pertussis toxin.

**Figure 33: Pertussis Toxin Inhibits 101P3A11 Mediated ERK Phosphorylation**



- Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.

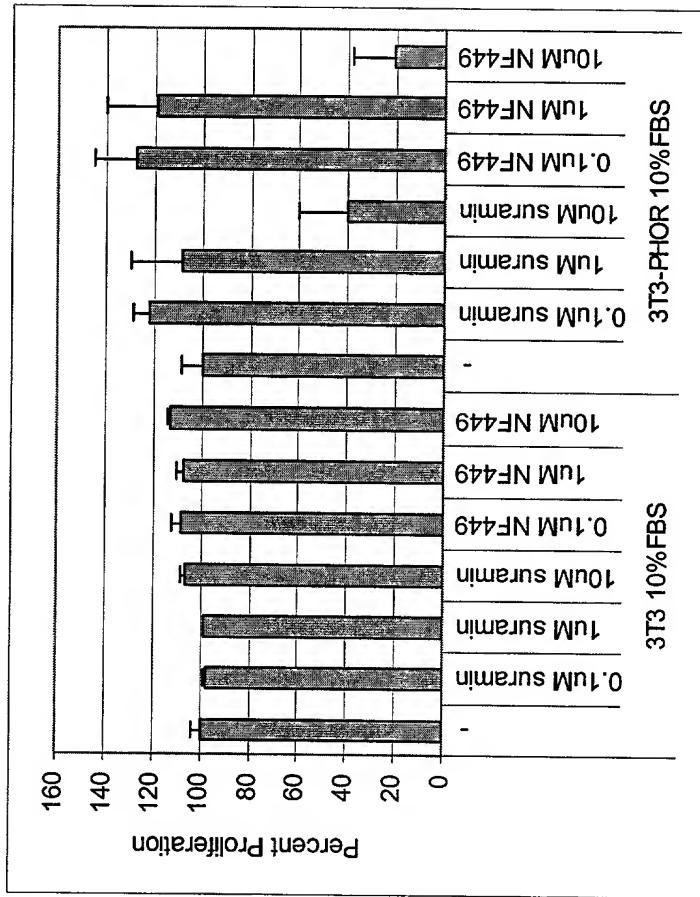
Figure 34: Pertussis Toxin Inhibited ERK Phosphorylation in PC3-101P3A11 Cells



• Pertussis toxin inhibited FBS- mediated ERK phosphorylation in 101P3A11 expressing cells.

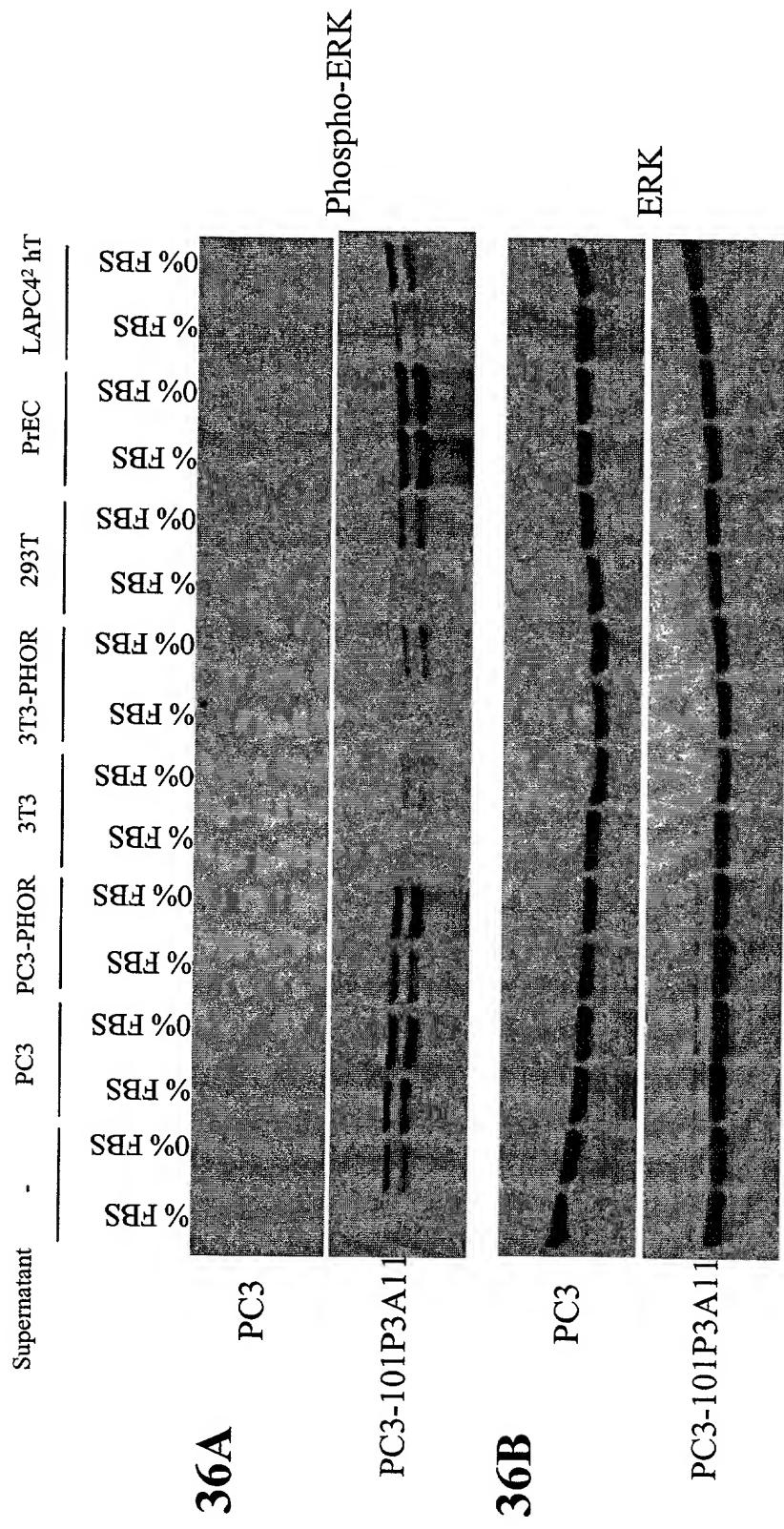
• The inhibitory activity of pertussis toxin on ERK phosphorylation was more dramatic in FBS-treated than EGF or GRP-treated PC3-101P3A11 cells.

Figure 35: Inhibition of 101P3A11 Mediated Signaling by Suranim



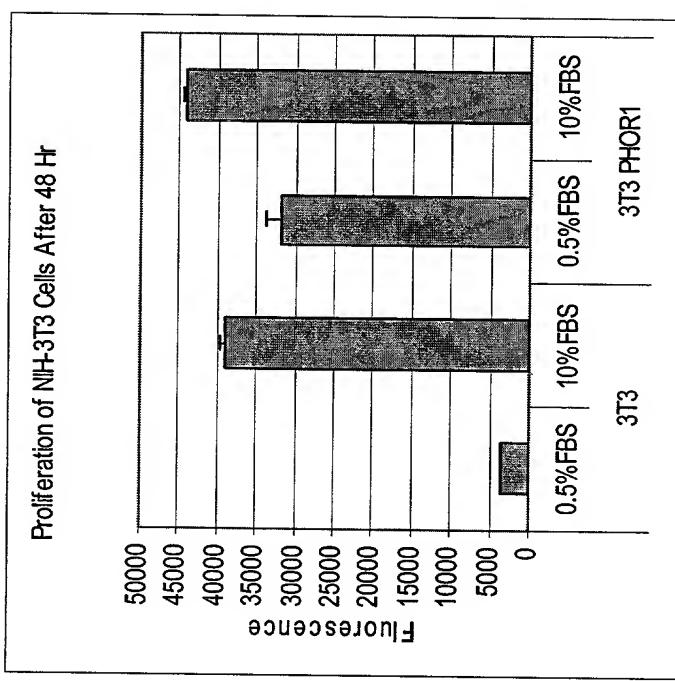
- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence of G protein inhibitors suranim and NF449. Proliferation was analyzed by Alamar blue after 72 hours.
- Suranim and NF449 inhibited the proliferation of 101P3A11 expressing but not control cells.

## Figures 36A-36B: 101P3A11 Mediated ERK Phosphorylation By Conditioned Media



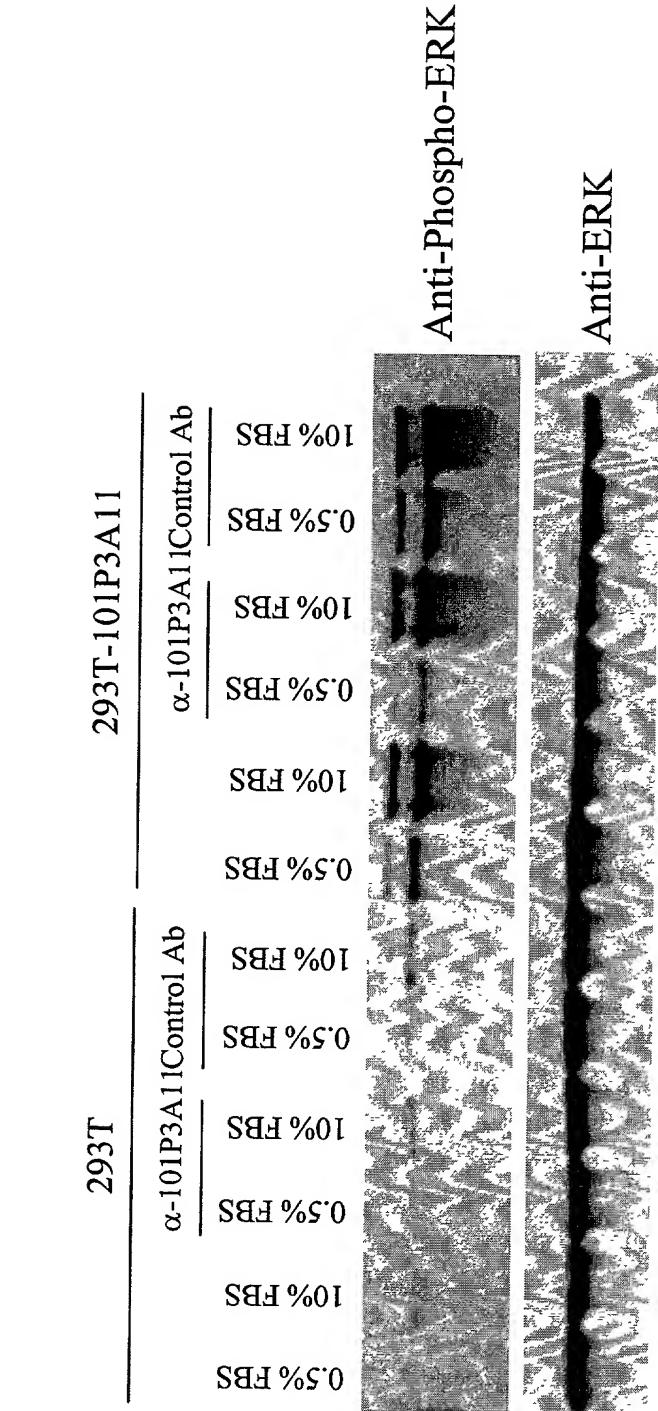
- Supernatants from PC3, PC3-101P3A11, PrEC and LAPC4<sup>2</sup> cells induce ERK phosphorylation in PC3 101P3A11 but not PC3 cells.
- Supernatants from 3T3 and 293T cells had little specific effect on ERK phosphorylation.

Figure 37: 101P3A11 Enhances The Proliferation of 3T3 Cells



- Control NIH 3T3 and 3T3-101P3A11 cells were grown in the presence of absence 0.5 or 10% FBS. Proliferation was analyzed by Alamar blue after 48 hours.
- Expression of 101P3A11 induced a 6 fold increase in the proliferation of 3T3 cells grown in 0.5% FBS.

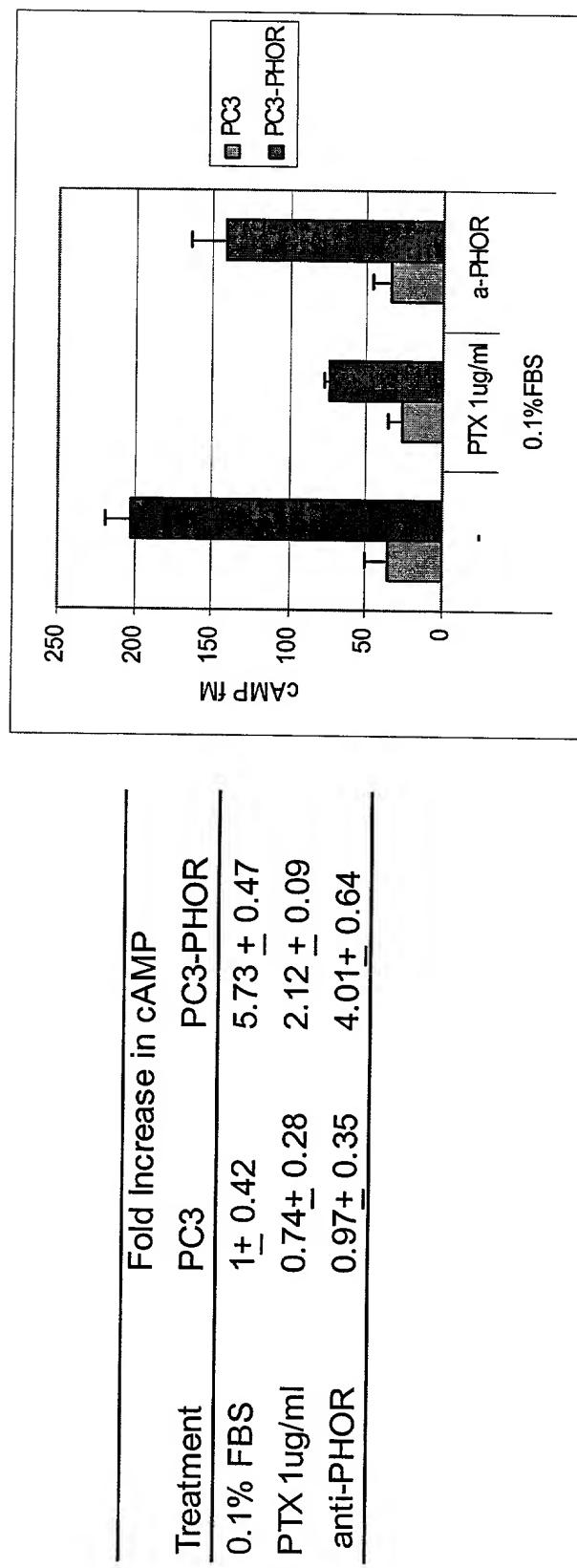
**Figure 38: Inhibition of 101P3A11 Mediated ERK Phosphorylation by 101P3A11 Specific Antibodies**



• Expression of 101P3A11 induced ERK phosphorylation in 293T cells.

• Anti-101P3A11 pAb inhibited ERK Phosphorylation in 293T-101P3A11 cells.

Figure 39: Anti-101P3A11 Ab Mediated cAMP Accumulation in PC3-101P3A11 Cells



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- Control PC3 cells and cells expressing 101P3A11 were treated with anti-101P3A11 pAb for 2 min and evaluated for intracellular cAMP content.

- The assay was performed in duplicate.

Figure 40A-40F

Fig. 40A. Prostate Cancer, 400X

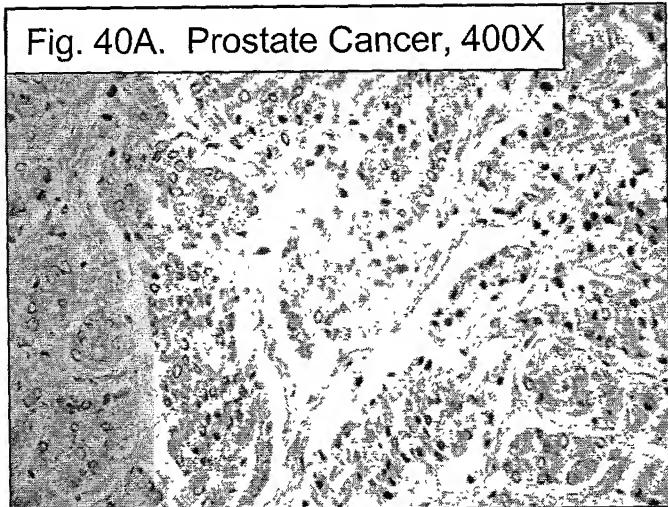


Fig. 40D. LNCaP, 400X

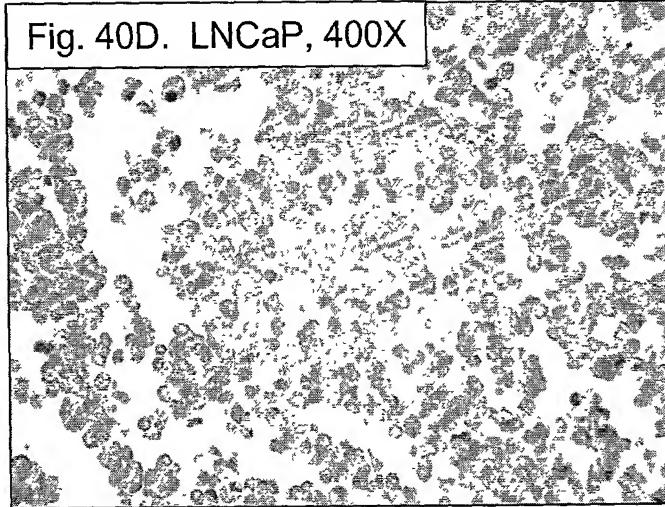


Fig. 40B. Prostate Cancer, 400X

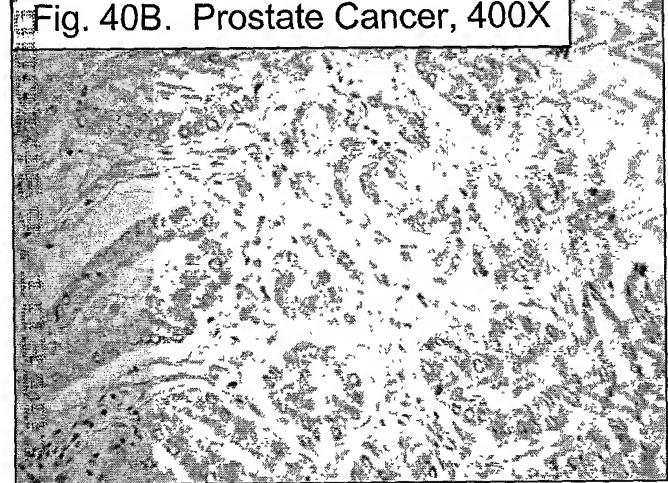


Fig. 40E. Prostate, 400X

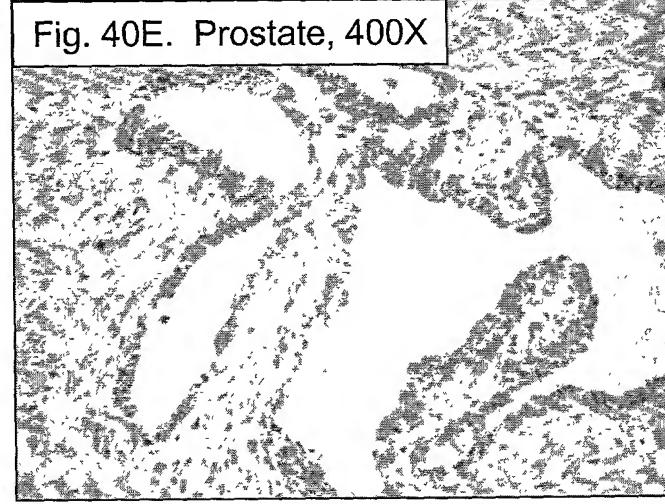


Fig. 40C. Prostate Cancer, 2000X

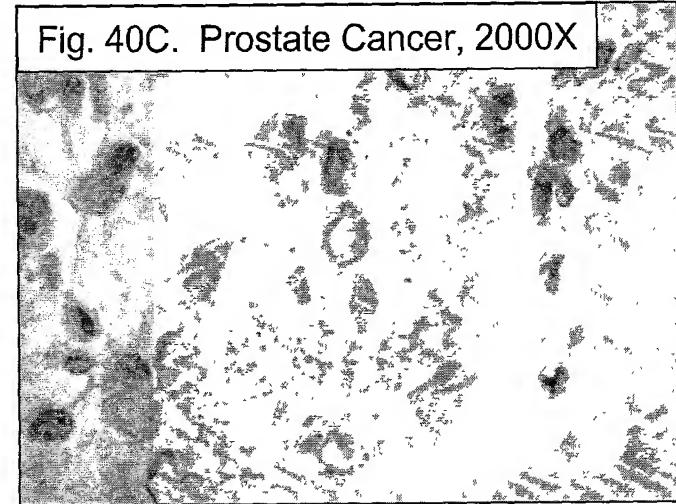
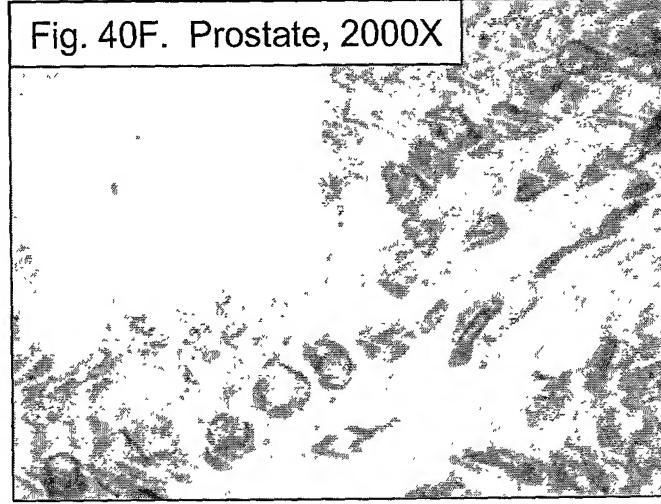


Fig. 40F. Prostate, 2000X



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Fig.41A Prostate Cancer, 800X

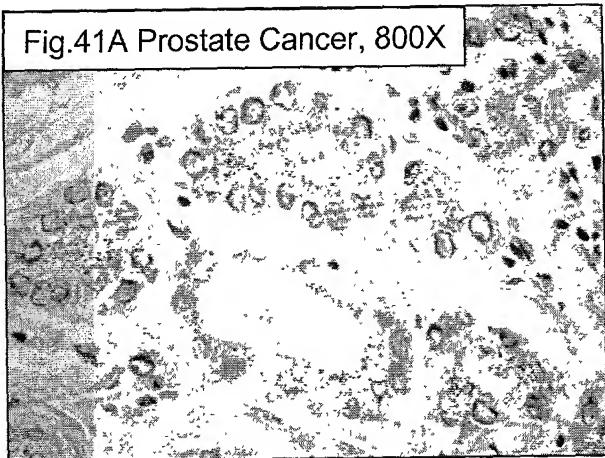


Fig.41B Bladder Cancer, 800X

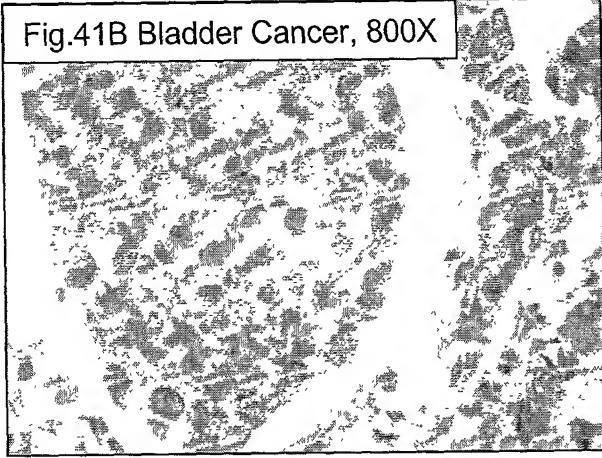


Fig.41C Kidney Cancer, 800X

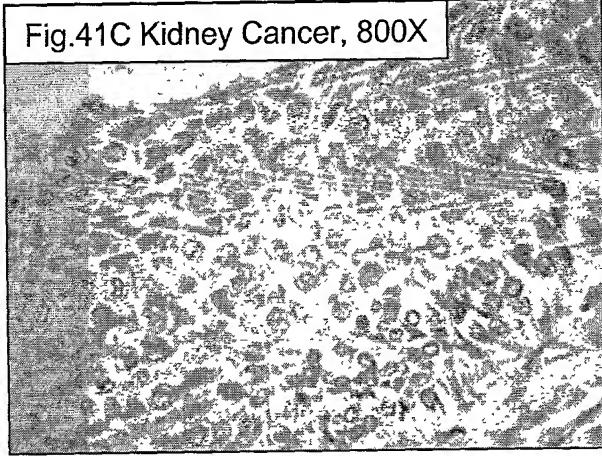


Fig.41D Colon Cancer, 800X

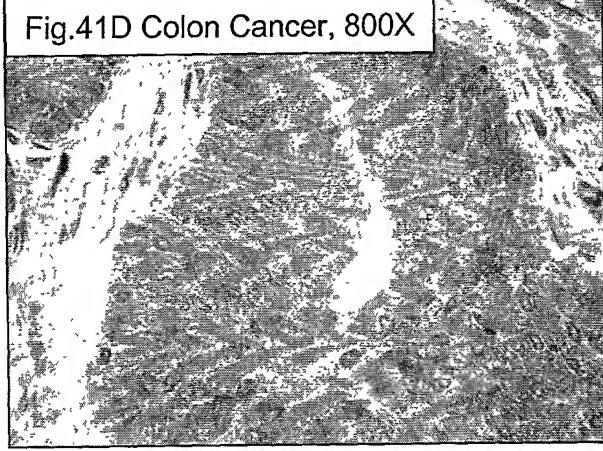


Fig.41E Lung Cancer, 800X

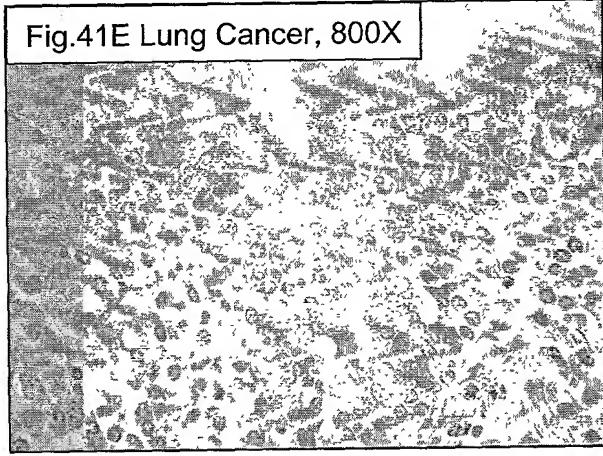
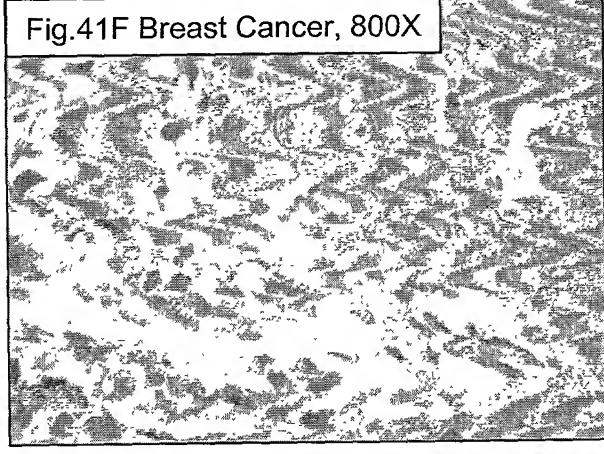
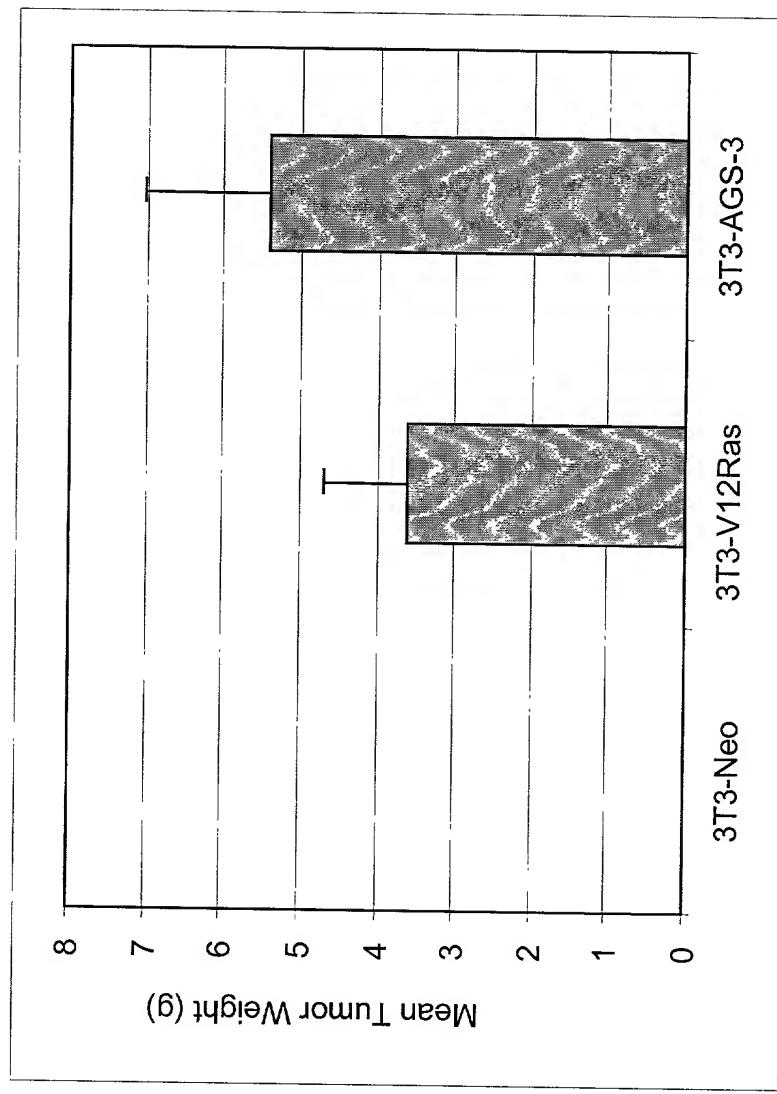


Fig.41F Breast Cancer, 800X



# Figure 42



# Figure 43

